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PLANT AND SOIL

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THE EFFECT OF SONIC VIBRATION ON THE BACTERIAL PLATE COUNT OF SOIL *

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INTRODUCTION

The inadequacy of existing plating techniques for estimating the numbers and proportions of microbial types in soil has long been realized [1]. Errors in estimates are attributable to, (a) the fact that no one plating medium can possibly provide a suitable growth environment for such a diversified population as that found in soil and, (b) the lack of a really efficient means of disintegrating soil samples so as to produce a uniform suspension of organisms in the diluting fluid. Furthermore, since micro-organisms adhere to soil particles, the number of bound organisms and therefore the number of organisms detected, will vary with the degree if flocculation of a soil. The accuracy of a plate count with a given medium will depend largely on the efficiency of the procedure adopted (in the preparation of dilutions) to break up soil particles and to dislodge these adhering organisms.

Gamble *et al.*[2] and Orcutt [4] have investigated the effectiveness of various diluents in dispersing soil aggregates and have shown that deflocculating agents such as sodium chloride and sodium metaphosphate materially increased the numbers of micro-organisms appearing on plates. The effectiveness of these agents was further increased when hand-shaking of the soil dilutions was replaced by high speed stirring in a Waring blendor [2].

In 1923, Whittles [7] described a vibration technique for the

* Contribution No. 447.

mechanical agitation of soil suspensions. In this technique dilute soil suspensions were passed over a diaphragm which was vibrated through a small amplitude at high frequency by a mechanism similar to that of an electric bell. Whittles reported enormous increases in numbers of organisms appearing on plates prepared from the „vibrated" dilutions. In a review of this work, Thornton [6] pointed out the impossibility of some of Whittles' results (probably due to inadequate sterilization) but indicated, in view of the obvious dispersion of the soil particles, that the method warranted further attention.

The similarity in principle between the apparatus devised by Whittles and present-day sonic oscillators has prompted a re-examination of the use of high-frequency vibrations in the preparation of soil suspensions for plate counting.

MATERIALS AND METHODS

Preparation and analysis of soil suspensions

Five grams of soil were placed in the sample cup of a 10-Kc Raytheon magnetostrictive oscillator and 50.0 ml sterile water added (equivalent of 1 : 10 dilution). After treatment at peak frequency for selected intervals 10.0 ml of the soil suspension was transferred to a sterile 90.0-ml dilution blank. Serial dilutions were then prepared in the usual manner by shaking for one minute between subsequent dilutions.

Numbers of micro-organisms in non-sonic-treated samples (control series) were determined by shaking the initial 1 : 10 dilution by hand for five minutes and subsequent dilutions for one minute. One-ml amounts of the appropriate dilutions were plated with soil-extract agar (S.E.A.) for the enumeration of bacteria and actinomycetes, and with Rose Bengal agar for fungi.

Nutritional classification of bacterial isolates

Nutritional grouping of bacteria isolated from representative plates was carried out using the technique of Lochhead and Chase [3] as later revised [5]. Nutritional groups G and S were omitted from these studies.

EXPERIMENTAL RESULTS

Numbers of bacteria, actinomycetes and fungi

In the course of these studies two soils designated as X and NG were subjected to high-frequency vibrations as described above for 1, 2, 3, 4, 5, 10 and 20 minute intervals. The numbers of bacteria,

actinomycetes and fungi as determined by plate counts after treatment are illustrated in Fig. 1.

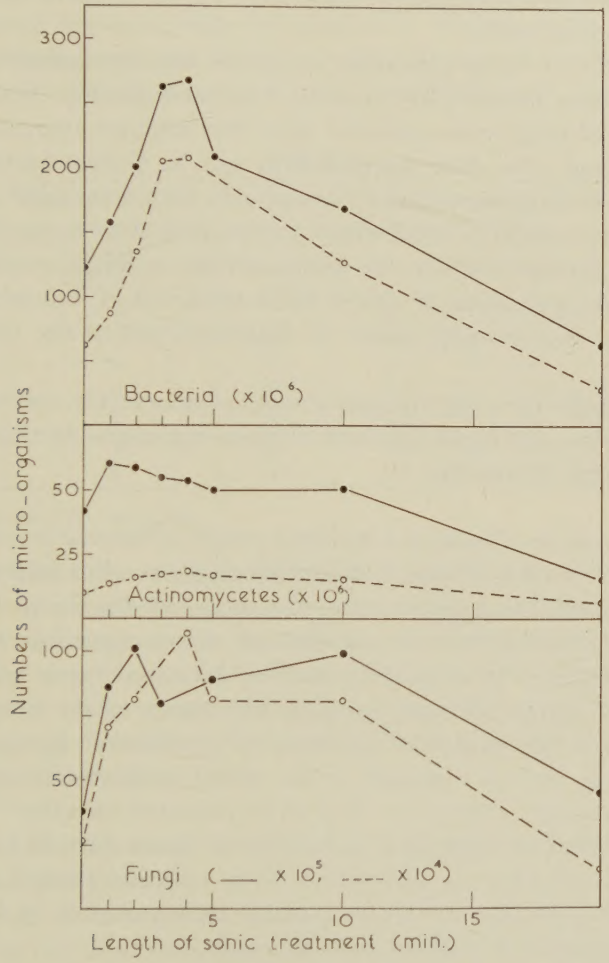

Fig. 1. Numbers of bacteria, actinomycetes and fungi from two soils after sonic treatment of the initial plating dilutions for varying intervals (——— Soil NG, – – – – – – Soil X).

In both soils bacterial numbers increased immediately from '0' time (no sonic treatment), reaching a peak after four minutes vibration. Soil X displayed the greatest increase in numbers (350%) at this point, while soil NG showed a somewhat lesser effect (250%).

Further exposure to sonic treatment beyond four minutes resulted in a gradual decrease in numbers of bacteria in both soils. After twenty minutes numbers of organisms were found to be lower than in the control series.

Numbers of fungi appearing on plates also showed striking increases when initial dilutions were treated sonically. The highest numbers of fungi were recorded after two minutes treatment with soil NG and after four minutes with soil X. Unlike bacteria, the numbers of fungi remained at a relatively high level until after ten minutes treatment beyond which a gradual decline in numbers was noted. It is suspected that the initial increase in fungal numbers was due to disaggregation of spores while retention of numbers at the high level was brought about by fragmentation of the vegetative growth.

Only slight increases in numbers of actinomycetes were noted in these studies and again numbers of these organisms decreased after ten minutes' treatment.

Effect of sonic treatment on nutritional groups of bacteria in soil

The observed increases in bacterial numbers after four-minutes' sonic treatment suggests that the vibration has effectively disrupted microbial colonies in soil and released micro-organisms from the soil aggregates. It is unlikely that all bacterial types will be released in equal portions; presumably there exists considerable variation in the degree of attachment of different species to soil particles as well as variation in the ability of bacterial colonies to withstand sonic vibrations. It is to be expected therefore that the balance between organisms isolated from plates derived from non-sonic-treated dilutions and isolates from sonically treated dilutions will vary markedly. It is also possible that variation in length of sonic treatment will affect such a balance.

In order to evaluate these possibilities some 300 colonies were isolated from soil-extract agar plates prepared from initial soil dilutions treated sonically for four and ten minute intervals. Isolates were also made from a control series of plates prepared by the usual handshaking procedure. One hundred isolates from each of the three series of two soils were classified according to their nutritional requirements. The results of these tests are presented in Table I. With soil X, increasing the length of sonic treatment

decreased the percentage of organisms falling into groups B, A and AG. Numbers of organisms in group Y remained relatively unaffected while a large increase in YS organisms occurred. Similar results were obtained with the isolates of soil NG where groups B and AG again decreased, groups A and Y remained unaffected and the relative numbers of group YS organisms increased.

TABLE I

Effect of sonic treatment of initial soil-plating dilution on nutritional classification of bacterial isolates

Soil	Sonic treatment (min)	Total organisms	Nutritional Group				
			B	A	AG	Y	YS
X	0	100	14.0*	18.0	51.0	8.0	9.0
	4	96	3.1	7.3	42.7	7.3	39.6
	10	93	2.2	4.3	31.1	10.8	51.6
NG	0	84	10.7	21.4	25.0	16.7	26.2
	4	105	4.8	15.2	33.3	19.0	27.7
	10	87	4.6	25.3	14.9	18.4	36.8

* Percent of total organisms

It is evident from these data that isolates from sonically treated dilutions show a marked difference in the balance between nutritional groups as compared to the isolates of untreated soil. As sonic treatment is increased the percentage of organisms which are less exacting in their nutritional requirements decreases while the percentage of those requiring complex growth factors, yeast and soil extracts increases.

Effect of sonic treatment on survival of pure cultures of the different nutritional groups in soil

Although an increase in numbers of micro-organisms takes place during the first four minutes of sonic treatment, the rapid decline in total numbers on further exposure suggests that harmful effects also occur. It is possible that while absolute numbers are increasing, some organisms are being destroyed by sonic vibration or by the grinding action due to the violent agitation of the solid soil particles during treatment.

To investigate this further, pure cultures of soil isolates were reintroduced into sterile soil and treated sonically for four- and ten-minute intervals. Two isolates of each of the five nutritional

groups were cultivated in YS medium, washed, resuspended in water and standardized turbidimetrically. One ml of a suspension of each of these isolates was added to 5.0 g of sterile soil in the chamber of the oscillator along with 50.0 ml sterile water. After four- and ten-minutes' sonic treatment aliquots were removed, diluted further and plated in the usual manner with S.E.A. At the same time identical suspensions of the combined organisms of each group were added to dilution bottles containing 5.0 sterile soil and 50 ml water. This control dilution (0 time) was hand shaken for 5 minutes and then plated.

Table II illustrates the degree of resistance of members of the

TABLE II

Effect of sonic treatment on isolates of different nutritional groups in soil

Nutritional group	Treatment in time minutes		
	0	4	10
B	20.1*	7.8	1.6
A	15.3	15.9	15.9
AG	19.8	13.7	7.2
Y	19.1	19.8	21.1
YS	17.7	18.4	10.0

* Bacterial numbers in billions

five nutritional groups to sonic treatment in the presence of soil. It is immediately apparent that the nutritionally simple group-B organisms offer very little resistance and are rapidly destroyed even by the four minute treatment. Slight reduction in numbers is also noted with the AG organisms while the remaining groups remain unaffected after four minutes. Numbers of surviving organisms of three of the five groups (B, AG, YS) decline rapidly on further exposure to the sonic treatment.

DISCUSSION

In the foregoing studies an attempt has been made to evaluate sonic treatment as a means of dispersing samples in the first dilution of a plating series for the enumeration of soil micro-organisms. A two- to fourfold increase in bacterial count was obtained by treating the first dilution for a period of four minutes while a sharp drop in numbers occurred on further exposure.

By means of a nutritional classification for the arbitrary characterisation of the soil population, it has been shown that a decided shift in bacterial types resulted from the exposure of soil dilutions to sonic vibrations. The observed shift in proportions of various types of bacteria in the population was presumably due to the combined effects of (a) the varying resistance of different micro-organisms to dispersal, and (b) their resistance to sonic vibration and attrition by soil particles. Control experiments indicated that while the nutritionally simple group-B organisms were reduced in numbers on four-minute treatment in soil, the numbers of the more complex groups were relatively unaffected. Since the B-group organisms represented only a small proportion of the total population it is felt that the shift in balance between groups was primarily due to disaggregation with a resultant increase in numbers of the more complex organisms. The sharp decline in numbers after ten-minutes' treatment was also noted in the pure culture studies where numbers were considerably reduced in most of the nutritional groups.

These results illustrate the importance of an aspect of plate counting which is frequently ignored. It is generally assumed that, although numbers obtained on plates do not represent the total number of organisms in soil, they are relative and as such can be used for comparative purposes, so that the ratio of any one type of organisms to another as it appears on plates is representative of the balance of these organisms in soil. These studies have shown that the numerical balance between groups changes on increasing dispersal of the initial soil dilution which suggests that the numbers and types of bacterial isolates from plates prepared in the usual manner are not representative of the population as it occurs in soil. The true numerical relationships of one group to another can only be determined when complete dispersion of all bacterial cells has been brought about without a simultaneous loss in organisms due to the severity of treatment employed for dispersion.

The procedure adopted in these experiments is not recommended as a routine technique although it is felt that organisms appearing after four-minutes' treatment are more representative of the actual equilibrium in soil than those obtained with the usual plating techniques. The method fails in that some organisms are being destroyed, even before complete dispersion of all bacteria has been

accomplished, though it is possible that this may be overcome by lowering the frequency and lengthening exposure time.

SUMMARY

Treating the first dilution of a soil-plating series to high-frequency vibrations resulted in initial increases in the numbers of bacteria, actinomycetes and fungi appearing on plates. Maximum numbers of bacteria were recorded after four-minutes sonic vibration. Further treatment resulted in a rapid decline in numbers. The nutritional classification of isolates from sonic and non-sonic treated soils indicated marked changes in the ratios between the nutritional groups after four- and ten-minutes' vibration.

ACKNOWLEDGEMENT

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THE EFFECT OF SOIL DRYING ON HUMUS DECOMPOSITION AND NITROGEN AVAILABILITY

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INTRODUCTION

Preliminary experiments with the macro-respirometer (Birch and Friend [3]) showed that when a soil was successively dried and rewetted decomposition after each rewetting followed a uniform pattern (see, for example, Figs. 1 and 2). Repetition of a similar pattern has since been found to occur under field conditions (Fig. 5) and this has important consequences on humus decomposition and nitrogen mineralisation. The effect of climate in relation to this pattern is discussed later.

One of the main problems in this work has been to explain why so little of the decomposable humus complex is decomposed after each rewetting since adverse physical (moisture, aeration) or chemical (toxic products) conditions appear not to be involved. It was tentatively concluded (Birch and Friend [3]) that successive dryings effected, on each occasion the release of small amounts of decomposable material from within the clay lattice where it was protected from microbial attack. Another possibility was that drying increased the amount of humus subsequently going into solution following which it was rapidly decomposed. More organic material can generally be extracted from a dry soil than a moist one and several workers (see Chase and Gray [6]) have ascribed the flush of decomposition that follows the wetting of a dry soil to this. From the experiments to be described it appears that these hypotheses are untenable. Humus decomposition is shown largely to involve direct microbial attack of the solid substrate, with repetition of the decomposition pattern depending on repetition of a similar

bacterial cycle, involving on each occasion a proportion only of the total utilisible substrate. Possible mechanisms to explain this behaviour are discussed.

EXPERIMENTAL

The macro-respirometer used in the following experiments has already been described (Birch and Friend [3]). Its sensitivity and simplicity of construction and operation render it particularly suitable for studying decomposition in soils for periods ranging from hours to months. Soils retain their mosture, and oxygen is automatically replaced as it is used up, the volume being simultaneously shown by a burette reading. About 20 minutes are required to record the daily rate of decompositions of 36 samples or treatments, and to reset the apparatus. The respirometer vessels were maintained at 25°C. The oven-dried soils were inoculated with 0.1 g fresh soil on rewetting but this appears to have little extra effect on subsequent decomposition compared with non-inoculation. Presumably the surviving population largely governs the subsequent course of decomposition.

Experiment 1. *The effect of air-drying and rewetting on decomposition*

Three 50-g samples of air-dry soil (6.2% C, 0.6% N) were brought to field capacity and put in the respirometer. After 10 days soils A and B were again air dried, rewetted and returned. The treatment was repeated twice more with A and once more with B after which B was oven dried for 24 hours at 100°C and rewetted. Soil C was used as a control to calculate the extra carbon mineralised after the 2nd, 3rd and 4th rewetting of A and B.

The pattern of decomposition (smoothed out) is shown in Fig. 1, and Table I summarises data not shown there.

TABLE I

Soil	Stage	Extra C mineralised mg/100 g air-dry soil	k
A	2	10.0	0.23
	3	10.0	0.24
	4	11.0	0.20
B	2	7.0	0.25
	3	11.0	0.26
	4	80.0	0.21

The k values were calculated from the equation $k = 1/t \log a/(a - x)$ where a is the gas uptake for the 5 day period after rewetting and x the uptake for t days (up to 5). For t = 1, 2, 3 and 4 the k values (averaged in Table I) for each flush of decomposition were reasonably constant indicating that the course of respiration followed a first-order reaction.

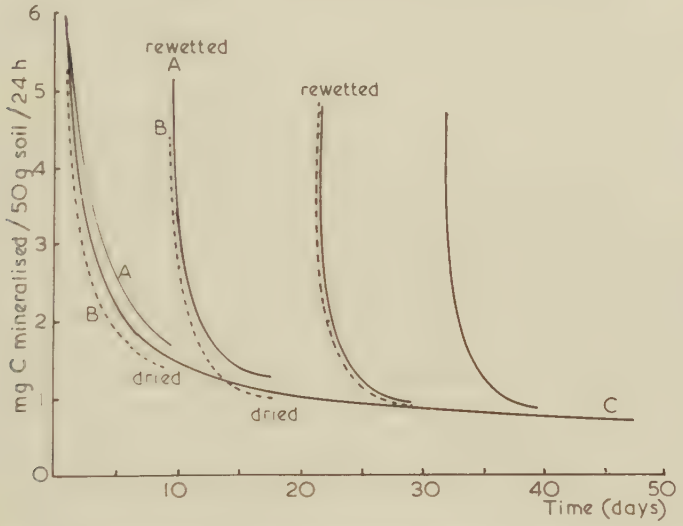

Fig. 1. The pattern of decomposition following each successive air-drying and rewetting of a soil.

The rapid decline in the rate of decomposition is not due to a shift in pH since this was found to be negligible. The development of toxic products could be responsible, but this appears unlikely (Expt. 11). Moreover washing the soil to remove possible soluble toxic compounds does nothing to enhance decomposition. When the course of decomposition follows a first-order reaction the concentration of substrate is often the limiting factor. Subsequent experiments show, however, that subtrate concentration, *i.e.* organic material in the soil solution is not involved. The rapid fall-off in the rate of decomposition could be due to adverse conditions in the respirometer. When, however, soil samples were taken from the respirometer exposed to the atmosphere and then returned there was no subsequent increase in the rate of decomposition. Moreover aeration and moisture conditions in the respirometer are virtually ideal.

Experiment 2. *To determine how often the drying and rewetting effect can be successively repeated.*

35 g of oven-dry soil (6.4% C, 0.7% N, pH 6.4) were brought to field capacity (15 ml water) and put in the respirometer. When the flush of decomposition was over the sample was again oven dried at 100°C for 48 hours and the procedure repeated. The results so far are shown (smoothed out) in Fig. 2. Oven-drying was employed because the magnitude of decomposition following this is greater than after air-drying (see Expt. 1) thus reducing the number of treatments required completely to exhaust the decomposable carbon reserve.

After the 43rd oven-drying and rewetting about 38 per cent of the total soil carbon has been oxidized, with a gradual decline, now levelling off, in the magnitude of each successive decomposition. The k values for each flush of decomposition (calculated as in Expt. 2) ranged in a random fashion from 0.22 to 0.27. The ratio of carbon to nitrogen mineralised during the first 18 treatments was 17.5 : 1. The magnitude of decomposition on rewetting was independent of the stage at which the previous drying took place (Fig. 2, points R and R'). This experiment is still proceeding to determine what proportion of the total soil carbon can be decomposed by this method.

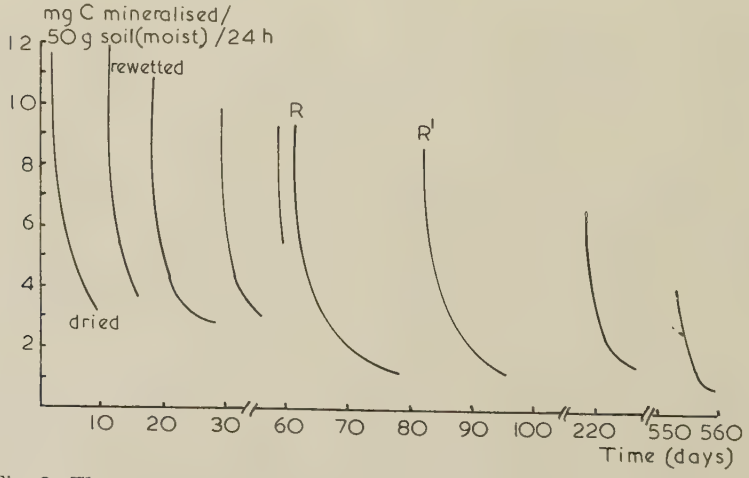

Fig. 2. The pattern of decomposition following successive oven-drying and rewetting (34 intermediate curves omitted).

In a parallel experiment using an acid soil (4.2% C, 0.24% N, pH 4.8) the magnitude of each successive flush of decomposition

fell off much more rapidly, and after the 12th treatment was very small. At this stage the carbon in the soil was reduced to 3.80 per cent indicating that only about 9.5 per cent of the original carbon was of a decomposable nature. The remainder must refer to undecomposable residues accumulated under acid conditions in the field. Microbial activity may also be reduced at the low pH but this did not affect decomposition after the first dryings and rewettings and it appears most probable that the residual material is inert to microbial attack.

Experiment 3. *To determine if successive oven-drying and rewettings, in the absence of intermediate decomposition, has a cumulative effect.*

40 g of oven-dry soil (A) were brought to field capacity and put in the respirometer. After 8 hours, and before decomposition began, the soil was removed and oven dried. This procedure was repeated twice more after which the soil was again oven dried, rewetted and allowed to decompose. A duplicate sample (B) was heated for periods equal to A (but without intermediate wetting) and then rewetted. Soil A therefore received four drying and rewetting treatments and Soil B one.

The magnitudes of decomposition for the six-day period after rewetting were A 54 mg C, B 53 mg C 100 g soil, a non-significant difference.

This experiment shows that the flush of decomposition following the wetting of a dry soil is not due to the effect of drying on the physical or chemical properties of the organic substrate, otherwise a cumulative effect would be expected. Drying affects the course of decomposition on rewetting rather than the decomposability of the substrate. This state of dryness is unaffected by previous dryness and rewettings and soils A and B start from the same state when allowed to decompose. Similarly with points R and R′ in Fig. 2 (Expt. 2). Here, however, R′ is slightly lower than R because of the slightly lower carbon content of the soil at this stage.

Experiment 4. *The effect of drying and rewetting on mineralisation of nitrogen.*

(a) Air-drying.

Three 40-g portions (B, C and D) of air-dry soil (7.7% C, 0.60% N) were brought to field capacity and put in the respirometer. After 10 days B was extracted for the determination of ammonia nitrogen and nitrate nitrogen

using a slight modification of Richardson's[17] method. Samples C and D were air dried at about 25°C for 2 days and returned to the respirometer. After 10 days C was extracted as for B, while D was again air dried, rewetted and returned to the respirometer. 10 days later this was extracted.

The results are given in Table II expressed on an air-dry basis.

TABLE II

Sample	No. of treatments	N in soil, ppm	
		NH_4–N	NO_3–N
A	0	30.0	182.0
B	1	10.0	202.0
C	2	10.0	222.0
D	3	10.0	244.9

Each air-drying gives, after rewetting an increment of about 20 ppm nitrate nitrogen. Assuming an acre 6″ of soil to weigh two million pounds this is equivalent to about 200 lb. of sulphate of ammonia per acre.

(b) Oven-drying.

This experiment was similar to the above, with oven-drying (100°C for 24 h) substituted for air-drying.

The results (Table III) are expressed on an air-dry basis, and the last column refers to the amounts of carbon and nitrogen mineralised during the breakdown period.

TABLE III

Sample	No. of treatments	N in soil, ppm		Ratio of C to N mineralised
		NO_3–N	NH_4–N	
A	0	167	29	
B	1	151	202	9.4
C	2	161	343	8.3
D	3	161	428	14.1

C : N ratios ranging from 10 to 20 have frequently been obtained with other soils for carbon and nitrogen mineralised during the active decomposition period following air- and oven-drying. Oven-drying greatly enhances nitrogen mineralisation on rewetting compared with air-drying.

Experiment 5. *The effect of different bases on humus solubility and decomposition.*

100-g portions of soil (6.5% C, 0.6% N, B.E.C. 30 me/100 g soil) were converted to the Ca, Mg, K and Na soils by shaking with 250 ml of *N* $CaCl_2$, $MgCl_2$ *etc.* The soils were then filtered under suction, washed with 200 ml of water and air dried for 48 hours. 30-g samples of each were then treated with 10 ml water and 1 g of fresh soil (as an inoculum) and put in the respirometer. Separate 10-g samples were extracted with 40 ml of water, and total nitrogen and mineral nitrogen were determined on the filtrates. Duplicate 30-g and 10-g samples were dried at 100°C for 24 hours and then treated as above except that 12 ml of water were added prior to putting them in the respirometer. After 16 days in the respirometer the samples were removed and again air dried (7 days at 25°C), or oven dried (24 hours at 100°C). The soils were then rewetted as before and put in the respirometer for 10 days. The results are summarised in Table IV. Organic nitrogen refers to total nitrogen (Kjeldahl's method) minus mineral nitrogen (direct distillation of extract with magnesium oxide) per 10 ml of extract. Milligrams C decomposed (1) is for 30 g soil after the 1st wetting and 16 days incubation and (2) after the 2nd rewetting and 10 days incubation.

TABLE IV

Group	Air-dry				Oven-dry			
Base	Ca	Mg	K	Na	Ca	Mg	K	Na
pH	6.30	6.30	6.60	7.09	6.29	6.33	6.77	6.91
mg soluble org. N	0.15	0.12	0.26	0.44	0.21	0.20	0.40	0.43
mg C decomposed (1)	34.3	40.7	38.6	35.9	46.1	56.8-	44.5	48.2
mg C decomposed (2)	15.6	15.4	15.1	17.2	30.5	40.2	31.5	33.6

These results show that while more organic material can be extracted from an oven-dry than an air-dry soil this is not related to the greater decomposition that occurs after oven-drying. Thus, between groups, the oven-dry Ca and Mg soils have less than half the amount of soluble organic material as in the air-dry Na soil but their magnitudes of decomposition are much greater. Moreover within each group the range of solubilities is wide but the magnitudes of decomposition are fairly uniform. Since the organic-matter content of the soils is also uniform, being little affected by the different treatments it appears that the organic matter itself is decomposed and that preliminary solution in water is not involved. The decomposition differences within groups could be due to the effect of the bases on the physical condition of the soil and therefore the accessibility of the organic matter. The decomposition

differences between groups, which is not due to solubility or total organic matter, could be due to the improved physical conditions of the soil after oven-drying or to the effect of oven-drying on subsequent microbial activity.

Experiment 6. *The decomposition of aqueous soil extract.*

100 g of oven-dry soil (as in Expt. 2) were shaken with 200 ml of water and filtered. The filtrate contained 1.50 mg organic N per 100 ml. Half the filtrate was evaporated to quarter the volume. Separate 30-g portions of another soil (2.4% C, pH 6.4), air-dry, were then treated as follows: – A, 10 ml water; B, 10 ml soil extract; C, 10 ml concentrated soil extract, and then put in the respirometer. These volumes brought the soil to field capacity. As a control 10 ml of extract were added to 100 g medium-grade sand plus 0.1 g soil as an inoculum.

The magnitudes of decomposition for the 6-day period after wetting were A 4.7 mg C, B 5.2 mg C, C 5.5 mg C per 30 g soil. Decomposition in the sand was negligible.

It is evident that the flush of decomposition following the wetting of the dry soil in Expt. 2 is not due to the solution of organic material. If this were so B or C above should differ from A by over 20 mg. The extract itself, as shown by its behaviour in sand, appears to be too dilute for microbial activity. The relative uniformity of the values for A, B and C support Expt. 5 and indicate that the humus fraction itself is attacked.

Experiment 7. *The relationship between decomposition and the percent carbon in the soil.*

(a) Different soils. Fig. 3 shows the relationship, which is highly significant, between the mg C decomposed/100 g soil (Y) (for the four-day period following the wetting of the oven-dry soil) and the percent carbon in the soil (X) The equation relating the two is

$$Y = 4.3\,X + 1.15$$

There is one obviously discrepant soil which has not been included in calculating this equation. This is similar to the acid soil cited in the second part of Expt. 2 and contains a high proportion of undecomposable carbon. Otherwise these results are in general agreement with those of Bunt and Rovira [5] who found that the

amount of readily decomposable material was generally proportional to the total organic matter content of the soil.

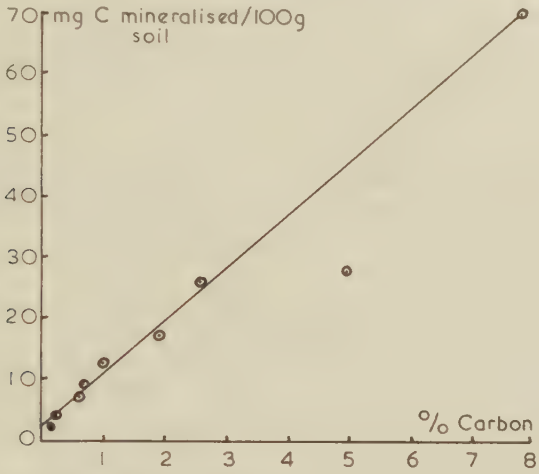

Fig. 3. The relationship between % C in the soil and the amount mineralised during the 4-day period after rewetting.

(b) Same soil. This soil has received various F.Y.M. additions over the last 26 years resulting in a range of values for percent C. Air-dry samples of the soils were brought to field capacity and put in the respirometer for 6 days. The resulting magnitudes of decomposition following very closely the percent C in the soil, are shown in Table V.

TABLE V

% C	mg C decomposed/100 g soil
0.920	8.00
0.962	8.29
1.196	13.93
1.214	14.54
1.410	19.03

The equation relating the two sets of values is highly significant.

$$Y \text{ (mg C decomposed)} = 23.1\ X \text{ (\% C)} - 13.6$$

The steeper slope of this regression line, compared with that for different soils, is probably due to the greater decomposibility of the humus derived from recently added F.Y.M.

Experiment 8. *The effect of wetting in two stages compared with one.*

35-g portions of oven-dry soil (similar to that in Expt. 2) were brought to 2.9, 5.7, 8.6, 11.4, 14.3, 17.1, 21.4, 25.7, 30.0 and 34.3 per cent moisture (field capacity) and put in the respirometer for 5 days. All the soils (except the last) were then brought at once to field capacity and returned to the respirometer for a further 5 days.

The patterns of decomposition are shown in Fig. 4, (with omissions to avoid overcrowding), while the magnitudes of decomposition for each of the two stages are given in Table VI.

TABLE VI

Moisture content, %	2.9	5.7	8.6	11.4	14.3	17.1	21.4	25.7	30.0	34.3
mg C decomposed (Stage 1)	1.8	2.4	3.0	4.2	9.0	14.4	21.0	24.6	27.0	33.6
mg C decomposed (Stage 2)	33.0	33.0	33.6	33.0	26.4	22.2	18.0	10.2	12.6	11.4
mg C decomposed (total)	34.8	35.4	36.6	37.2	35.4	36.6	37.0	34.8	39.6	45.0

It will be noted that the total magnitudes of decompositions are almost the same in all instances. The sample wetted in one stage to field capacity is a little higher than the others, but the trend of the graphs in Fig. 4 indicates that this superiority would be lessened had the samples been kept longer in the respirometer after the second stage wetting.

In this experiment decomposition during Stage 1 is directly proportional to the moisture content above 14.3 per cent, while decomposition up to 11.4 per cent moisture is small. It appears that only above a critical moisture content (in this instance about 11 per cent) is water available for microbial activity. The proportionality that then exists between decomposition and moisture content may reflect the ability of increasing moisture contents to support an increasingly large microbial population. When the soil is brought from sub-optimal moisture conditions (Stage 1) to optimal conditions (Stage 2) the restriction on the maximum population possible no longer obtains and the deficit in decomposition is then made good. It is likely that under field conditions (Expt. 10) the flush of decomposition at the start of the rains will be of the same

magnitude whether the soil is brought at once to field capacity or whether this is achieved somewhat gradually.

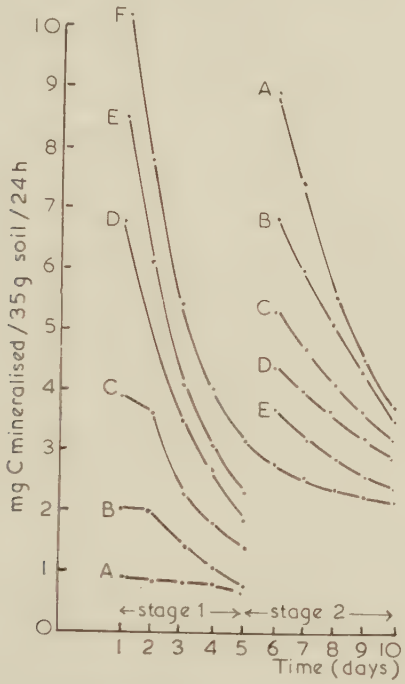

Fig. 4. The pattern of decomposition following two-stage wetting. Soil F at field capacity (34.3% moisture) throughout. Soils A, B, C, D, and E, stage 1, contained 11.4, 14.3, 17.1, 21.4 and 25.7% moisture; on the fifth day they were brought to field capacity.

Experiment 9. *The decomposition of leaf in subsoils and sand, and the subsequent effect of drying and rewetting.*

Two subsoils were selected with the following characteristics.

Soil	Depth	%N	%C	% Clay	me/100 g soil		pH
					T.E.B.	B.E.C.	
10612	22–39″	0.040	0.11	54.4	17.6	23.3	5.8
11612	10–23″	0.064	0.48	32.0	4.8	9.2	5.7

25 g were mixed with 50 g of medium-grade, acid-washed sand, brought to field capacity and treated with 0.4 g of powdered cotton leaf (40.0% C, 4.0% N). 75 g of sand alone (S) were similarly treated, and the samples put in the respirometer.

Rapid decomposition occurred in each instance, falling to a slow and almost steady rate after about 10 days. A further 0.4 g of leaf was then added, with similar results. Altogether five additions were made in this way. After each of these similar patterns of decomposition occurred, the magnitude however increasing with successive additions. This is shown in Table VII, where mg C decomposed refers to the 9 day period following the addition of 0.4 g leaf (160 mg C). Table VII shows also the overall decomposition after the 5 additions of leaf.

TABLE VII

Sample	mg C mineralised after additions						mg C added + mg C in sample	Decomposed %
	1	2	3	4	5	Total		
10612	40.9	67.2	75.4	85.9	98.2	367.6	828	44.4
11612	60.3	71.0	75.4	94.6	101.9	403.2	920	43.8
Sand	68.0	73.8	70.6	75.4	90.2	378.0	800	47.3

There is little to choose between the decomposition occurring in the different media.

The samples were then air dried at 20–25°C for 18 days after which they were restored to field capacity and put in the respirometer. The familiar flush of decomposition occurred in all instances. When this was over the samples were dried at 100°C, with similar results on rewetting, and these occurred again after a further oven-drying and rewetting. This experiment is still proceeding. The results so far given are in Table VIII and refer to the mg C mineralised during the first 6 days after rewetting. The corresponding k values are also given. Treatments 1, 2, 3 refer to the first, second and third drying and rewettings.

TABLE VIII

Sample	mg C mineralised after treatments			k values after treatments		
	1	2	3	1	2	3
10612	25.0	18.6	13.2	0.18	0.17	0.23
11612	24.4	14.4	13.2	0.21	0.17	0.22
Sand	15.0	18.6	12.0	0.20	0.22	0.21

The magnitude of the drying effect decreases following successive treatments which indicates a decline in the amount of material

involved. The k values, however, remain fairly constant and are similar to those reported in previous experiments for decomposition following the wetting of a dry soil. The uniformity of behaviour with both sands and soils indicates that the drying effect is little affected by the medium in which the organic matter is present.

Experiment 10. *To determine the seasonal course of decomposition in the field.*

The macro-respirometer is very suitable for measuring the magnitude of decomposition occurring in the field at any particular time. Thus, a soil put in the respirometer immediately after sampling will remain under conditions similar to those in the field with regard to the microbial population and moisture, particularly during the early period in the respirometer. The main difference between respirometer and the field conditions is that of temperature which, in the respirometer, remains steady at 25°C. In comparative studies, however, as in the present experiment, this is of minor importance.

A plot of fallow virgin land, which had recently been under wattle and a subsequent volunteer growth of grass was selected. It contained 0.66% N and 6.0% C. Immediately after sampling the soil was passed through a 2-mm sieve and 50 g placed in the respirometer. A duplicate sample was used for moisture determination. The magnitude of decomposition was then followed in the respirometer and the average daily oxygen uptake for the first two days (expressed as mg C in Fig. 5) was taken as a comparative measure of the rate of decomposition occurring in the field at the time of sampling.

Sampling started at the beginning of a wet season and continued at intervals of a few days throughout a series of alternate wet and dry periods. The results are shown in Fig. 5, and refer to soils containing 30 per cent moisture in the field at the time of sampling, thus putting the data on a comparable basis since the magnitude of decomposition also varies with the moisture content of the soil.

The main feature of interest is the marked decrease in the rate of decomposition in the field as the wet season continues, and the recovery in the rate of decomposition when rain falls again after a dry period. This conforms with the behaviour of soils on wetting and drying under laboratory conditions and reveals an interesting and important pattern of decomposition under natural conditions.

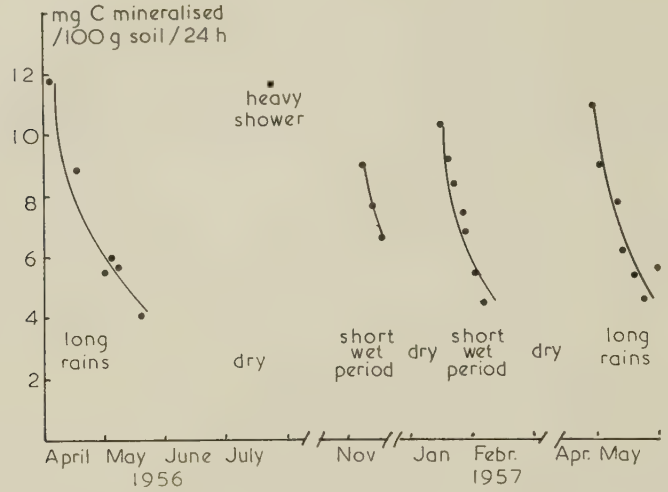

Fig. 5. The seasonal pattern of decomposition in the field.

Experiment 11. *To test for the development of toxic conditions during decomposition.*

Three 35-g portions A, B and C of oven-dry soil (as in Expt. 2) were each brought to field capacity with 15 ml water and put in the respirometer. After 6 days, when the flush of decomposition was over, a further 10 g of soil were brought to field capacity and added to A, and 20 g, similarly treated to B, Sample C received no additional treatment and served as a control. The soils were then kept for a further 6 days in the respirometer.

The results are summarised in Table IX.

TABLE IX

Sample	mg C decomposed			
	1st 6 days	2nd 6 days	2nd 6 days minus control	Column 4 calculated to 35 g
A	26.3	18.0	8.0	28.0
B	26.8	27.0	17.0	29.8
C	26.0	10.0	0	

It is seen from a comparison of columns 2 and 5 that the magnitude of decomposition that follows the wetting of a dry soil is not affected by the presence of soil in which decomposition has already occurred. The pattern of decomposition was also unaffected. Evi-

dently decomposition is not accompanied either by development of toxic conditions or of a microbial population inimical to those active during the early period after rewetting, otherwise the values for column 5 should be less than those for column 2. The effect of drying or subsequent decomposition cannot then be ascribed to destruction of toxic conditions or the elimination of one group of micro-organisms that competes with another.

DISCUSSION

This paper deals mainly with the decomposition of humus rather than of macro organic matter (leaf litter *etc.*). Since humus accounts for by far the largest proportion of soil carbon and nitrogen its decomposition behaviour is of particular importance. One of the main factors governing decomposition is the drying and wetting cycle of the soil the frequency and effectiveness of which is determined by climate and various agricultural practices. Some aspects of this cycle will now be discussed in relation to (a) nitrogen availability, (b) humus decomposition and (c) the mechanism involved.

a. Nitrogen availability. Under both laboratory and field conditions when a dry soil is moistened a characteristic pattern of decomposition occurs, as in Figs. 1 and 2. The magnitude of decomposition largely depends on the percent carbon in the soil and the conditions (hot or cold) under which the soil is dried. Decomposition is accompanied by nitrogen mineralisation (Expt. 4). Winsor and Pollard [18] also found that air-drying soils generally increased the amount of carbon and nitrogen subsequently mineralised, and various reports in the literature confirm this.

Maximum nitrate production will, then, occur at the start of the rains particularly in tropical soils subject to well defined wet and dry seasons. Once this initial flush of decomposition is over a period of slow decomposition and nitrogen mineralisation sets in. Early planting, an empirical procedure widely used by the peasant cultivator in East Africa, aims at having the crop planted when the rains begin. This practice, which has much to commend it, ensures that the crop gets part at least of the nitrate produced during the flush of decomposition that then occurs. Some of the nitrate is subsequently leached away and will not be available to

crops planted later in the rainy season, which usually make poorer growth although moisture is adequate.

This pattern of nitrogen mineralisation has an obvious bearing on soil-fertility studies and emphasises the dynamic aspect of nitrate supply. This is greater at the start of the rains than later on and, with some soils, may shift from an adequate to an inadequate level. Conventional methods for determining available soil nitrogen take no account of this decline. In addition to a changing rate of supply during a wet period there will be seasonal differences, depending on rainfall, in the amount of nitrogen mineralised in a given soil. Low rainfall which is generally more intermittant than high rainfall will be associated with a greater frequency of the drying and wetting cycle and therefore with greater nitrate production. In this connection nitrogen responses in East Africa, where these occur, are more common in wet than dry years. Similarly Glover [9] has reported that maize yields decline as seasonal rainfall increases above about 30 inches a year. Further, any circumstances that affect the intensity or prolongation of soil drying prior to the main rains, such as dry-seasons rains, will affect the amount of nitrate then produced. Rainfall is therefore an important factor to consider in soil-fertility studies and in the interpretation of the results of nitrogen-fertiliser trials. An appreciation of the pattern of nitrate production may also be of value in irrigation where, to some extent, the drying and wetting cycle can be controlled.

Any practice that intensifies soil drying should lead to more nitrate production when the rains begin. The beneficial effect of soil burning is related to this, and Experiment 2 shows that each of several successive burns should be effective. Analyses here have shown that burning has very little direct effect on humus destruction so that loss of humus due to burning depends largely on the increased decomposition that follows when burned soils are wetted. Since this is usually small in relation to the total humus reserve successive burnings should be effective over many years provided that physical deterioration of the soil and surface erosion do not set in.

The fact that humus can undergo marked decomposition for only a relatively short period (Expts. 1 and 10) in spite of its inherent decomposability helps to explain the persistence of nitrogen in the soil. The general lack of response to nitrogen in the

wheat-growing areas of Kenya is referable to this and Cornish [7] has reported similar behaviour for the stoney and mallee soils of the South Australian wheat belt. These observations agree with the pattern of decomposition already described in which the amount of material decomposed in any one cycle is generally small in comparison with the total amount present, but significant as regards the amount of nitrogen mineralised. The prolonged breakdown of this fraction compared with the rapid decomposition of the organic matter (leaf residues *etc.*) from which it is derived may partly account for the residual responses to organic manures obtained on the sandy tropical soils at Ukiriguru, Tanganyika. (Peat and Brown [14]).

The importance of drying on soil fertility has been reported, among others, by Lebedjantzev [12]. It appears from present studies that nitrogen mineralisation following the wetting of a dry soil is one of the main factors involved. Mineralisation of organic phosphorus can also occur but probably to a much smaller extent and in this instance fixation by the soil can also take place. The amounts of bases and trace elements (*e.g.* manganese) that go into solution are also affected by drying. Here the effect is due to physical rather than biological changes in the soil, but it serves to emphasise the dynamic aspect of soil fertility already mentioned, where conditions at the start of the rains, with regard to nutrient supply, are very different to those obtaining later in the wet season.

b. Humus decomposition. Expt. 10 shows that under field conditions a relatively high rate of decomposition occurs when a dry soil is wetted falling later to a slower and more constant rate as moist conditions continue. This pattern is repetitive and the frequency with which it occurs will be an important factor in the rundown of soil carbon. Since low rainfall, as pointed out earlier is usually associated with a greater frequency of wet and dry periods than high rainfall it should, above a certain lower limit, be conducive to an accelerated loss of soil carbon. A fairly general and direct relationship should therefore obtain between rainfall and the carbon, or total nitrogen, content of soils. In this connection Birch and Friend [4] found that the regression equations for total nitrogen on rainfall were remarkably alike for East African and North American (Middle West) soils. Another example is the similarity between the total nitrogen and humidity factor relationship found by Jenny [10]

for North America, and Prescott [16] for Australia. No doubt these relationships also involve the direct effect of rainfall on vegetative growth and subsequent humus production, but their marked similarity in spite of different temperature and vegetation conditions indicates the operation also of a common factor, the wetting and drying cycle, on humus decomposition.

Any agricultural practice that enhances soil drying such as burning, exposure by ploughing, wide spaces, intertilled cropping or bare fallowing should hasten the loss of soil carbon and numerous reports confirm this (see, for example, Mann and Barnes [13]). On the other hand crops affording shade will tend to conserve carbon. The high carbon level of constantly moist soils may also be due, partly, to the absence of the wetting and drying cycle. Anaerobic conditions cannot always be held reponsible for provided the soils are not waterlogged aerobic decomposition can occur, as pointed out by Allison *et al.* [1].

In addition to the pattern of decomposition mentioned above a third stage may be recognised under field conditions, namely the almost complete absence of decomposition under dry conditions when microbial activity is virtually negligible. The cycle of decomposition where wet and dry seasons prevail is therefore the simple one shown in Fig. 5 where relatively rapid decomposition (Stage 1) is succeeded by slower decomposition (Stage 2) under wet conditions and by virtually no decomposition (Stage 3) when dry conditions are restored.

c. The mechanism involved. The uniform pattern of decomposition following each successive drying and rewetting also occurs in a sand medium (Expt. 9). In other experiments not reported in the text it has also been found to occur with plant material alone and with commercial dried yeast (which usually contains added organic substrate). The presence of soil is therefore not essential to the drying effect, nor is the rapid decline in the rate of decomposition after rewetting necessarily due to physical changes in the soil (*e.g.* swelling of the colloids with alteration of the pore space) or inactivation of enzymes by adsorption. Since solubility of humus is also not involved (Expt. 5) the problem is more or less restricted to the effect of drying, or heating, on microbial behaviour.

Spores are resistant to adverse conditions such as drying and heating and their subsequent germination and multiplication after

each successive drying and rewetting should follow a fairly uniform pattern. A prominent feature of the early phase of a bacterial culture (*i.e.* the phase of physiological youth) is high metabolic activity which is very much greater than that of later generations developing after the period of maximum multiplication. This activity is characterised by high oxygen uptake and carbon-dioxide and ammonia production. Enzyme activity, an important factor in the breakdown of solid substrate, is also at a maximum during the early stages of growth as shown by Wooldridge and Glass [19]. The high initial rate of decomposition could, then, be due to the high metabolic activity associated with physiological youth, this activity declining as the culture ages and passes into the resting stage. A sequence of this behaviour would be expected each time a soil is dried and rewetted, for with the amount of substrate a more or less constant factor (though gradually declining) one has, on each occassion, a repetition under virtually uniform conditions of a bacterial cycle involving similar micro-organisms, namely those that survive the drying or heating treatments. With a given soil the magnitude of each successive flush of decomposition will decline as the organic reserve diminishes. With different soils the magnitude will depend largely on percent carbon (Expt. 7). Provided each drying leaves spores for germination and multiplication on rewetting the magnitude of decomposition should be independent of the number of times the soil is wetted and dried without intermediate decomposition (Expt. 3) and of the stage at which the soil is dried during the preceding flush of decomposition (Expt. 2) though here the carbon lost during the preceding flush will slightly reduce the magnitude of that following.

The rapid decline in the rate of decomposition after each rewetting cannot be explained by substrate exhaustion, since substrate is present in an amount considerably in excess of that required for a single flush of decomposition. It also appears improbable, from what has been said in Expt. 1, that the development of toxic conditions in the medium is responsible. Porter [15] states that the reasons for the slowing-down process in a bacterial culture are not well understood at present and factors other than mere exhaustion of the food supply and the accumulation of toxic metabolic products appear to be involved. Bail [2] has suggested that the crowding of the bacteria in a culture may cause the cessation of growth, there being

a maximum level of population that the medium can support. Such conditions could conceivably be reached in the limited volume of soil solution available for the bacteria. Bail considered that bacteria destroyed by heat do not consume the available biological space, thus permitting the recurrence he observed of the bacterial culture cycle in the same medium after heating, a behaviour similar to that after oven-drying and rewetting a soil.,

A further possibility is that the rapid decline in the rate of decomposition is due to the fact that micro-organisms active in the decomposition of substrate just after wetting have soon to compete with later-developing less active ones. This would infer the presence of several resistant species which, on rewetting interact on each occassion in a remarkably uniform manner (Expt. 2) conforming approximately to a first-order reaction. Evidence for such behaviour is contra-indicated by Expt. 11 which shows that the population present when the flush of decomposition is over does nothing to interfere with the normal pattern of decomposition of an oven-dried soil when this is introduced. Chase and Gray [6] found that when a dry soil was rewetted respiratory activity conformed to two superimposed first-order reactions. From this they concluded that after a dried soil has been remoistened there is present in addition to the more slowly decomposable humus complex a limited supply of readily available water-soluble organic matter and that this is responsible for the initial flush of decomposition on rewetting. It is however doubtful from Expts. 5 and 6 if water-soluble material plays a significant rate in this respect. The decline in the rate of decomposition appears, in the present work to be due to a decline in microbial activity. It is not possible to state the reasons for this decline, but it may be relevant that the logarithmic death phase in a bacterial culture also conforms to a first-order reaction, as does the decline in the rate of decomposition as shown by the k values in Expt. 2. The period over which these were calculated may, however, include part of a succeeding phase which would account for the fact that the first-order equation is not exactly followed

It has frequently been observed in these studies that prolonged air-drying results in greater decomposition on rewetting than short air-drying although the precentage loss of water is almost the same. Also vacuum-drying for five days over phosphorus pentoxide was hardly more effective than air-drying over the same period

in spite of the greater dehydration of the soil. Vacuum-drying and oven-drying were almost the same as regards moisture losses, but oven-drying resulted in a much greater magnitude of decomposition on rewetting and was as effective at 60° C as at 100° C. Since drying does not affect the inherent decomposibility of humus (Expt. 3) the drying effect must be due primarily to microbial changes. Curran and Evans [8] postulate that following heat shock spores are stimulated to intense metabolic activity, which could partly explain the marked decomposition that follows oven-drying. The enhanced effect of prolonged over short air-drying is difficult to explain. It is apparently not due to the increasing elimination of a competing microflora as the soil dries out since, without drying, this has no effect on the decomposition of added soil (Expt. 11). It is conceivable that prolonged drying progressively affects the physical conditions of the soil (shrinking of colloids *etc.*) leading to a greater exposure of organic surface for subsequent microbial attack. In this case vacuum-drying would be expected to have effects of similar magnitude, but this does not occur. In discussing the question of heat shock Knaysi [11] says that the effect has been attributed to the elimination of vegetative cells among which may be undesirable or weak variants. A similar result may be obtained with prolonged air-drying. Whatever the mechanism toxic conditions and substrate solubility seem definitely to be ruled out. The drying of soils appears largely to involve changes in microbial behaviour and this aspect of the problem is now being further studied.

The similarity in decomposition patterns under laboratory and field conditions indicates similar microbial behaviour in the field though here the situation is somewhat complicated by the simultaneous decomposition of leaf litter *etc.*, which was sieved out in the laboratory samples. The general pattern of behaviour is however almost as uniform and repetitive as under laboratory conditions and can usefully be applied to problems involving humus build-up and decomposition, and nitrogen mineralisation.

SUMMARY

Respirometer experiments show that when a dry soil is moistened a characteristic pattern of decomposition occurs in which an initial period of relatively rapid decomposition (stage 1) falls, during a few days, to a slow steady rate (stage 2). This pattern is repetitive with successive dryings and rewettings

and is common to all soils so far investigated. The magnitude of decomposition depends in the percent carbon in the soil and on the drying conditions, air-drying being less effective than oven-drying. Decomposition during Stage 1 conforms approximately to a first-order reaction and proportionate amounts of nitrogen are mineralised. A similar pattern of decomposition occurs under field conditions throughout successive wet and dry seasons.

Evidence is presented to show that decomposition involves direct microbial attack of the solid organic substrate and that the recurrent pattern of decomposition is due to the state in which the microbial population is left after drying and its subsequent behaviour on rewetting. The rapid decline in the rate of decomposition on rewetting (Stage 1) appears not to involve (1) the development of toxic conditions, (b) physical changes in the soil (since similar patterns of decomposition also occur with organic material alone or in sand) or (c) rapid decomposition of organic material made soluble by drying.

The operation and repetition of this pattern of decomposition in the field has important consequences in the rundown of soil carbon and the mineralisation of soil nitrogen particularly where well-defined wet and dry seasons occur. These consequences are discussed in relation to climate and certain agricultural practices.

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EFFECT OF FARMYARD MANURE ON THE TRACE ELEMENT CONTENT OF SOILS AND OF PLANTS GROWN THEREON *

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INTRODUCTION

In a previous publication [1], it was shown that farmyard manure contains appreciable quantities of trace elements, and reference was made there to reports of instances where applications of manure had reduced or eliminated disorders due to deficiences of boron, manganese and zinc. Because little specific information is available, an experiment was conducted to study the effect of farmyard manure on the trace-element content of soils and of plants grown thereon.

MATERIALS AND METHODS

Cultivated-surface samples from six soil types found in Carleton County, Ontario, were selected; Uplands sandy loam, St. Thomas fine sand, Castor silt loam, Grenville loam, Bearbrook clay and Carp clay loam. The trace-element content of the manure used, expressed in parts per million of dry material, was: boron, 26.2; manganese, 123.0; cobalt, 1.06; copper, 23.9; zinc, 100.3; molybdenum, 3.1.

Five pounds of air-dry soil were placed in each of 12 glazed, half-gallon pots (two for each soil type). Dried manure was added to the soil in one pot of each soil type, at a rate of 20 tons of moist manure per acre, using the average moisture content of 76 per cent determined in previous work. After thorough mixing, water in an amount equivalent to half the water-holding capacity of the dry soil was added to all 12 pots. The pots were kept on a shelf in the laboratory and were weighed once a week, water being added where necessary to maintain constant weight. Throughout this experiment the water used was distilled water re-distilled from pyrex flasks.

* Contribution No. 377, Chemistry Division, Science Service.

At two-month intervals, the soil in each pot was removed and thoroughly mixed. The initial application of manure was repeated at this time. Thus, at the end of 24 months, each manured pot had received the equivalent of 240 tons of moist farmyard manure per acre.

Two months after the last manure treatment, a representative soil sample was taken from each pot for chemical analysis. The pots were then placed in the greenhouse where phosphatic and potassic fertilizers were added at rates equivalent to 200 lb. P_2O_5 and 200 lb. K_2O per acre. The pots were sown to ladino clover, which was harvested just before flowering stage on four successive occasions. Plant samples were air dried and the four cuts from a pot composited for analysis.

Chemical methods standard for this laboratory [8] were used. For molybdenum, soils were digested with $HClO_4 + HNO_3 + HCl$, extracted material dissolved in 6.2 *N* HCl, Fe and Mo removed by ether following 40 per cent citric acid, and Mo extracted with isoamyl alcohol after adding KCNS and $SnCl_2$. The final color was read in a colorimeter.

Dried plant material was charred, pulverized and extracted with HF for manganese determination, and with a mixture of HNO_3, H_2SO_4 and $HClO_4$ for determination of copper, cobalt, zinc and molybdenum. Total boron was determined by the turmeric method.

TABLE I

Trace-element content of manured and unmanured soils (as ppm air-dried soil)

Soil type and treatment	pH	Organic matter %	Nitrogen %	Boron H_2O sol.	Manganese			Cobalt, total	Copper, total	Zinc, total	Molybdenum, total
					Total	Ex.	Eas.-red.				
Uplands											
Unmanured	5.8	2.83	0.15	0.38	300	0.85	9.4	4.8	7.0	51.2	0.63
Manured	7.2	5.11	0.20	0.88	323	0.85	6.2	5.0	7.7	53.4	0.73
St. Thomas											
Unmanured	5.2	4.44	0.17	0.26	275	1.02	4.1	3.4	3.0	32.1	0.68
Manured	6.5	6.75	0.30	0.62	257	0.70	3.8	3.3	3.5	34.5	0.80
Castor											
Unmanured	6.1	4.07	0.13	0.39	324	1.93	10.3	3.9	4.1	28.8	0.93
Manured	7.2	4.83	0.23	0.81	308	1.83	8.0	4.1	5.0	37.3	0.91
Grenville											
Unmanured	7.3	4.00	0.24	0.43	1277	1.02	373.0	8.5	13.1	58.7	1.12
Manured	7.4	6.15	0.32	0.78	1242	1.72	321.0	7.8	13.9	62.7	1.25
Bearbrook											
Unmanured	4.7	4.58	0.19	0.25	370	6.25	8.4	9.2	15.5	61.7	0.91
Manured	5.8	6.97	0.29	0.75	335	1.93	10.3	7.8	16.0	63.3	0.97
Carp											
Unmanured	6.5	7.22	0.36	1.04	663	2.95	91.8	11.7	34.0	107.9	0.46
Manured	7.0	10.19	0.45	1.09	671	3.10	72.8	11.0	35.0	110.4	0.67
L.S.D. (*P* .05)	0.5	0.77	0.03	0.17	N.S.	N.S.	N.S.	N.S.	0.2	2.7	0.08

RESULTS

Table I gives the pH, percentages of organic matter and nitrogen, and trace-element content of the soils when they were sampled before applying P and K. Manure applications increased soil pH, organic matter and nitrogen percentages, and levels of water-soluble boron, total copper, zinc and molybdenum. These increases were significant at $P = 0.05$. There was no significant effect on levels of total cobalt or of total, exchangeable or easily reducible manganese.

Table II gives the yield of ladino clover and the quantities of trace elements it contained. The data are incomplete due to an insufficient amount of material in some cases. Two cuts only were harvested from the St. Thomas manured soil. Application of manure resulted in increased yields.

TABLE II

Soil type and treatment	Soil pH	Weight of four cuts		Trace element content of ladino clover (as ppm air-dried material)					
		Green g	Air dry g	Boron	Manganese	Cobalt	Copper	Zinc	Molybdenum
Uplands									
Unmanured	5.8	53.1	7.5	39.7	67.5		9.6	37.0	1.86
Manured	7.2	89.8	11.3	25.2	38.2		8.3	71.0	7.34
St. Thomas									
Unmanured	5.2	46.1	6.6	29.4	69.5				
Manured	6.5	19.5*	2.2*	23.7	23.0				
Castor									
Unmanured	6.1	85.0	13.1	35.5	49.0		7.5	156.0	0.96
Manured	7.2	135.0	15.9	26.7	25.2		6.8	64.5	2.23
Grenville									
Unmanured	7.3	96.7	13.7	30.0	85.8		7.3	58.0	4.20
Manured	7.4	187.3	22.7	28.2	66.5		7.8	101.0	4.10
Bearbrook									
Unmanured	4.7	77.6	9.2	25.7	152.5		8.4	76.0	< 0.05
Manured	5.8	155.2	16.0	31.2	39.2	0.17	8.4	90.0	1.35
Carp									
Unmanured	6.5	140.9	17.6	32.5	40.2	0.15	10.0	69.0	1.89
Manured	7.0	146.8	19.9	26.5	38.2	0.18	10.0	70.0	2.36

* Plants died after second cutting

Uptake of boron was less on manured than on unmanured soils, except for Bearbrook where the pH of the manured sample was below 6.0. The difference in the alkaline Grenville was small. Although

water-soluble boron in the soil was increased by manure, boron content of ladino clover was generally depressed. Uptake of boron appeared to be related to soil reaction.

Uptake of manganese was less on treated than on untreated soils. It was greatest on untreated Bearbrook soil which had a pH of 4.7. Uptake of manganese also appeared to be influenced by soil reaction.

Quantities of copper and zinc found in the clover showed no consistent variation with treatment and there was little difference in average values for manured and unmanured soils.

Uptake of molybdenum was greater on manured than on unmanured soils. The difference was quite pronounced on acid Bearbrook soil but negligible on alkaline Grenville. Again, uptake appeared to be related to soil reaction.

DISCUSSION AND CONCLUSION

The uptake of certain plant nutrients may be affected by available quantities of other nutrients. For example, Stout *et al.* [6] have demonstrated that large increases in the molybdenum content of plants may be induced solely through the application of phosphate fertilizer, Mulder [4] has shown an inhibiting effect of manganese on the uptake of molybdenum, and Shive [5] has referred to interrelationships between calcium and boron, and between potassium and boron, in plant nutrition. When a complex material such as farmyard manure is mixed with soil, there is the possibility of a considerable number of interrelationships but it would be very difficult to sort out the effect of one nutrient upon another. It is generally accepted, however, that boron and manganese are more readily available under acid conditions and that molybdenum is more readily available under alkaline conditions. Davies [2] referred to a number of papers emphasizing this point in regard to molybdenum, Mitchell [3] indicated that molybdenum is made more available by liming and that the uptake of boron is reduced by liming, and Toth and Romney [7] stated that the dominant factor affecting manganese uptake was soil pH. Since in this experiment manure applications increased soil pH and since the uptake of boron and of manganese was less, whereas the uptake of molybdenum was greater, on manured than on unmanured soils, it is

suggested that the influence of manure on trace-element availability may have been an indirect one due to its effect on soil reaction.

ABSTRACT

Farmyard manure, added to six soils at intervals over a two-year period until the equivalent of 240 tons of fresh manure per acre had been applied significantly increased pH values, organic matter and nitrogen contents water-soluble boron levels and total amounts of copper, zinc and molybdenum. The uptake of boron and manganese by ladino clover was less, and that of molybdenum was greater, on the manured than on the unmanured soils. It is suggested that the influence of manure on trace-element availability may be an indirect one, due to its effect on soil reaction rather than to any increased amount of these elements that may be added in the manure.

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THE INFLUENCE OF THE ENVIRONMENT ON SEED AND SEEDLING-MORTALITY

VII. DEPTH OF SOWING OF MAIZE

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INTRODUCTION

The depth at which a seed is sown in the soil may influence both its germination and its successful establishment as a seedling. At various distances below the surface the germination of a seed may be delayed or dormancy enforced by the local soil conditions – for example high concentrations of carbon dioxide in the deeper layers of the soil may inhibit germination, or the germination of light-sensitive seeds may be prevented by the absence of light in the soil. If germination occurs, the food reserves of deeply sown seeds may be insufficient to permit the shoot of the seedling to reach the soil surface or the seedling may be so weakened or damaged by the extended growth to the surface that its resistance to pathogenic attack or its subsequent powers of competition are seriously reduced. Seeds which are sown too shallowly may meet different hazards which prevent successful establishment. In particular the water relations of the soil fluctuate more widely at the surface than in the deeper layers, and seeds sown near the surface may often meet conditions suitable for germination but which are rapidly followed by periods when the surface layers become so dry that many seedlings die. Moreover, seeds at the surface of the soil are exposed to predatory birds, rodents and smaller pests while deep sowing offers a large degree of protection. In farming and gardening practise it is customary to sow large seeds deeper than small and this tendency may be correlated with the greater food reserves present in large seeds which make emergence from a

depth more successful and also with the fact that very few large seeds demand light for germination. However relatively few studies have been made of the influence of depth of sowing of crops on the rate and extent of field emergence.

Dungan [3] compared the emergence of a Corn Belt variety of maize (U.S. 13) which was not adapted to deep sowing, with the variety Navajo which was grown by the Navajo Indians who practised deep sowing. He found that Navajo gave 11 per cent emergence from 30 cm * deep sowings, 61 per cent from 25 cm, 67 per cent from 20 cm and 100 per cent from 10 cm. The corresponding emergence for U.S. 13 was 0, 0, 56 and 89 per cent. The seedlings of U.S. 13 were badly damaged when the grain had been sown deeply, suffering breakage of the coleoptile and damage to the leaves. The ability of Navajo to emerge from deep sowings was associated with its ability to develop a very long mesocotyl and to utilise all the endosperm reserves.

Andrew [1] studied the influence of depth of sowing on the stands and vigour of seedlings of sweet corn under controlled conditions of temperature. Using four inbreds and two of their hybrids, sown at a depth of 2.5 and 10 cm, he demonstrated that deep sowing contributed to poor stands in three ways:

(1) formation of permanent root formation near the coleoptilar node was delayed.

(2) the increase in length of first internodes and of time required for emergence increased both the zone liable to attack from soil-borne pathogens and the time during which such attack could occur (Hanf [6] had noticed in other species that long hypocotyls were more liable to damage by pathogens than short).

(3) The balance between the time of permanent-root formation and the time of decay of the mesocotyl was upset by deep sowing so that the absorptive capacity of the seminal roots was lost earlier.

Inge and Loomis [13] found that maize seedlings raise the stem apex to near-ground level by growth of the mesocotyl which is sufficiently plastic to allow response to depth of sowing, and Andrews demonstrated that a strain with an inherently short mesocotyl (inbred 5002) suffered heavy mortality from sowings made at 10 cm depth.

Black [2] has shown for subterranean clover (*Trifolium subterraneum*) that there is an optimal temperature (21°C) for the transference of food reserves from cotyledons in the extension of the hypocotyl and that both depth of sowing and temperature affect the weight of cotyledons at emergence – it is likely that similar relations hold for the elongation of the mesocotyl at the expense of endosperm reserves in maize.

Martin *et al.* [16] sowed sorghum grains at 1.2 cm, 4 cm, and 6 cm depth in pots of soil at 60 per cent of the moisture-holding capacity, and main-

* Where references quote depth in inches or temperature in °F the values have been transformed to centimetres and °C to give uniformity.

tained the pots at temperatures of 15, 20, 25, 30 and 35°C. They found that at the lower temperatures there was a reduction in emergence brought about by deeper sowing but that there was no significant effect at higher temperatures.

Erickson [4] compared the emergence of selected large, medium, and small seeds of lucerne (*Medicago sativa*) sown at 0.6 cm and 1.9 cm depth. The deeper sowing was slightly advantageous to the large seed, but became disadvantageous with decreasing seed size. Forbes and Ferguson [5], working with species of Zoysia found that emergence was reduced if the seeds were sown deeper than 0.6 cm and that the highest emergence was from surface sowings – but these were greenhouse experiments in which the soil surface was not allowed to dry out.

Although shallow sowings may make it possible for a seed with small reserves to emerge and to establish rapidly, there is a danger that such germinating seedlings may be killed by desication of the surface soil before a root system has been properly established. Such an effect was demonstrated by Harper [9] for the germination and establishment of seeds of *Ranunculus bulbosus*. Surface-sown seeds gave 66 per cent emergence, optimal emergence was obtained by sowing at 0.2 cm depth (83 per cent) followed by 43 per cent at 0.3 cm, and 41 per cent at 0.6 cm.

Heydecker [12] has reviewed other literature on the response of plants to depth of sowing and studied the effects of sowing depths from 0–9.5 cm on the establishment of cabbage, lettuce, carrots and onion seedlings. The optimal depth for all these species was in the range 1.5–2.5 cm. It was also established that date of sowing and sowing depth influenced the percentage emergence significantly and independently.

The present paper relates to the influence of depth of sowing on maize in laboratory, pot and field experiments.

EXPERIMENTAL METHODS AND RESULTS

For all experiments the variety of maize, White Horsetooth was chosen – the seed samples were obtained from Southern Rhodesia. This particular variety had proved especially sensitive to pre-emergence mortality, thus it could be expected to give a response to depth of sowing, moreover this variety had been used in previous studies of the influence of date of sowing, soil aspect and soil colour and the results were available for comparison (Harper [7 8] Harper *et al.*[10 11], Ludwig *et al.*[14 15]). The grain had been treated with a commercial preparation of tetramethyl-thiuram disulphide (T.M.T.D.) as a dry seed dressing so that there was some protection against seedling blight.

The soil used in both box and pot experiment was obtained from a field at the University Field Station, Wytham on which maize had been grown the previous year and in which the present field experiments were carried out. The soil is derived from Oxford clay overlying Thames gravel.

(a) *The influence of depth of sowing under conditions of controlled temperature*

Cardboard boxes (30 cm × 30 cm × 30 cm) were filled with soil: the boxes were divided vertically into 4 equal parts and in each quarter 100 grains were sown at 2.5, 5, 10 or 20 cm depth – each depth of sowing was replicated between boxes. The boxes were kept at 23°C in an incubator and the pots were watered regularly to maintain the soil at or near field capacity. The number of seedlings which had 'emerged' (*i.e.* the coleoptile was visible at the soil surface) was recorded daily and from the data two parameters were extracted (1) the *ultimate percentage emergence* which was the number of seedlings emerging expressed as a percentage of the germination capacity, (2) the *time for* 50 *per cent emergence* which was obtained from graphs of the progress of emergence and is expressed as the number of days from sowing until 50 per cent of the *ultimate* emergence was obtained.

In this experiment the most rapid and successful emergence was obtained from the shallowest sowing – see Fig. 1. Grains sown at

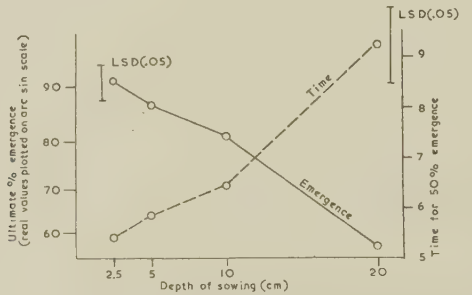

Fig. 1. The influence of depth of sowing on (a) *ultimate* % *emergence* (arc sin transformed data) and (b) *time for* 50% *ultimate emergence* (days), of White Horsetooth maize at a controlled temperature of 23°C.

20 cm gave only 73.5 per cent emergence and required 9.2 days to reach 50 per cent ultimate emergence compared with 90.5 per cent emergence from 2.5 cm depth in which case 50 per cent of this value was reached in 5.4 days. The seedlings emerging from 20 cm depth took longer to reach the surface than might be predicted from the behaviour of the shallower sowings.

(b) *The effects on maize emergence of sowing depth both in pots out of doors and in the field*

A field experiment was carried out in 1955. The land was ploughed in autumn and cultivated with a rotary cultivator in the spring. During the final preparation of the seed bed the surface was levelled so that there might

be no variation in the aspect of the plots (Ludwig *et al.*[14]). The experiment was of randomised-block design and included four sowing depths (2.5, 5, 7.5. and 10 cm), three sowing dates (April 6th, April 20th, and May 4th) and four replicates. The position of plots within each of four replicate blocks was randomised both for depth and date of sowing. Individual plots were 30 × 30 cm square and in each 50 grains were sown in five rows of ten grains. Depth of sowing was controlled by using a dibbing peg with an adjustable cross bar as a depth marker.

Concurrently with the field experiment a pot experiment was carried out. Grains from the same sample were sown in soil from the same site in plastic pots of 20 cm diameter. Before sowing, the pots were partly filled allowing for a further layer of soil on top which determined the depth at which the grains were buried: fifty grains per pot were distributed evenly on the surface and were covered. The pots were placed in the open, near the field plot and dummy pots were arranged around them to minimise the effects of direct irradiation of the sides. The pots were watered daily to maintain the soil at approximately field capacity.

As before in both experiments emergence of individual seedlings was recorded and the two parameters *ultimate percentage emergence* and *time for 50 per cent emergence* were determined.

From Table I it is evident that the *ultimate percentage emergence* of seedlings in both pots and field plots was greatly influenced both by date and depth of sowing. With one exception, the greatest number of seedlings emerging from any one sowing date was from sowings made at a depth of 2.5 cm. The *time for 50 per cent emergence* increased with depth of sowing. The most rapid emergence and the highest ultimate emergence are found for sowings made on April 20th; this date was followed by a three-week period of higher mean soil temperature than the other two sowing dates. In general, emergence from all depths and all dates was higher from the field plots than from the pots.

There was one striking departure from these general trends: grains sown on April 6th in the field at 2.5 cm depth gave a lower *ultimate percentage emergence* than from 5 cm in the field and lower than sowings made at 2.5 cm depth in pots (see † in Table I). This unexpectedly low value for emergence was associated with an abnormally long *time for 50 per cent emergence*. In the three weeks following April 6th rainfall was less than 1.2 cm and the surface soil was exceptionally dry, so that grains sown in the upper layers of the soil were restricted in their development by lack of moisture. Under such conditions on the bases of the findings of Harper [7 8], it

TABLE I

Comparison of rates and extent of emergence from different depths of sowing in pots and in field plots, 1955.

Sowing date	Sowing depth, cm	Pots		Field	
		% Ultimate emergence	Time for 50% ult. emergence	% Ultimate emergence	Time for 50% ult. emergence
April 6th 1955	2.5	65.0	18.6	60.0†	33.2
	5.0	41.0	24.8	69.4	20.5
	7.5	29.6	31.8	44.0	23.3
	10.0	9.0	34.7	41.0	27.2
April 20th 1955	2.5	91.0	14.0	91.6	23.0
	5.0	76.0	19.8	82.4	18.4
	7.5	52.0	23.7	74.0	21.3
	10.0	44.0	32.4	67.4	22.7
May 4th 1955	2.5	67.4	21.4	87.0	18.6
	5.0	31.4	25.5	64.4	20.0
	7.5	26.4	30.1	60.0	22.8
	10.0	6.4	31.1	36.0	25.3

Analyses of variance (arc-sin transformed data).

Source of variation	D.F.	Ultimate emergence		50% time	
		Pots	Field	Pots	Field
Blocks	3	*	*	*	—
Depth	3	***	***	***	***
Date	2	***	***	***	***
Depth × date	6	—	*	***	***

* = significant at $P = 0.05$
*** = significant at $P = 0.001$

would be expected that the mortality would be high when germination and emergence are delayed. A similar but less significant delay in emergence from the shallowest sowing, was also observed from grains sown on 20th April. There were no such anomalous results in the pot experiment because the pots were regularly watered.

The analysis of variance of the values for *ultimate* percentage emergence in this experiment shows a significant interaction between date and depth of sowing in the field experiments but not in the pots. However this interaction was smaller than the small block effect and was due almost entirely to the one anomalous result for April 6th. The interaction between sowing date and depth of sowing on *time for 50 per cent emergence* was greater and was highly significant (P 0.001) in both experiments.

It is interesting that the coefficient of variation in the field and pot experiments is of the same order (pot experiment 6.74 per cent

and field experiment 8.57 per cent). Very little was gained in precision by carrying out the more time-consuming pot studies. Moreover, the temperature conditions in the pots were much more extreme than in the field and so in this respect did not provide more controlled, but less controlled conditions than in the field. More precise comparisons of the soil temperature in pots and in the field were obtained by recording maximum and minimum temperatures at 2.5, 5, 7.5 and 10 cm depth in pots of soil similar to those used in the main experiment. Temperature readings were taken at 9 a.m. and the results for a six day period from June 17th to June 24th, 1955 are presented for depths of 2.5 and 10 cm in Figure 2.

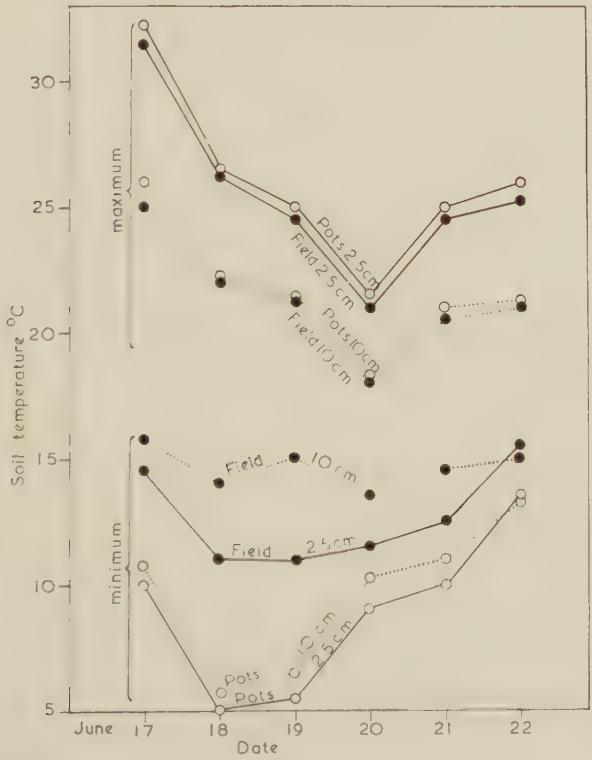

Fig. 2. Day-to-day variations of maximum and minimum temperature at 2.5 and 10 cm depth in pots of soil and in the field.

The maximum temperature reached in the pots during this period exceeded that in the field quite regularly, but at most by

2°C. The minimum temperature in the pots was 4–6°C lower than at the comparable depths in the field. The influence of depth on minimum soil temperature was almost completely eliminated in the pots.

The observed differences between temperature fluctuations in the field and in pots can of course be largely explained by the insulating effect of surrounding soil in the field plots. It seems likely, therefore, that differences in the mortality between field and pot conditions are due to the different temperatures experienced as well as to the higher soil-water content maintained in the pots.

(c) *The effects on maize emergence of depth of sowing under field conditions with and without supplementary watering*

In 1956 the depth of sowing experiment was repeated in the field with sowings again made on April 6th, April 20th, and May 4th but the range of sowing depths was extended to 2.5, 5, 10 and 20 cm. The design of the experiment was changed to include plots which were watered daily to bring the soil to field capacity; while the remainder only received normal rainfall (or in one case snow). Water was applied in amounts calculated according to the season and the weather conditions of the previous day (from tables kindly provided by the Meteorological Office of the Air Ministry). In this experiment grains were sown in specially excavated plots which were subsequently filled in to give the desired depth of sowing. This was considered preferable to the use of a dibbing peg which consolidated the soil under the grain. Values for *ultimate percentage emergence* and *time for 50 per cent emergence* were calculated from the progress curves for emergence.

The results are presented graphically in Fig. 3, and daily maximum and minimum soil temperatures at 2.5 and 20 cm throughout the experiment are shown in Fig. 4.

Striking differences are apparent between sowing dates, depths of sowing and between watered and unwatered plots. The highest emergence on the watered plots was obtained when the grain was sown at 2.5 cm and emergence decreased progressively with depth of sowing. The number of seedlings emerging from any given depth increased with successively later sowings, indeed sowings made at a depth of 20 cm on May 4th gave greater ultimate emergence than sowings made at 5 cm on April 6th. The influence of depth of sowing was greatest in the earlier sowings.

Interactions between date and depth of sowing were much more complex in the unwatered plots. The most successful establish-

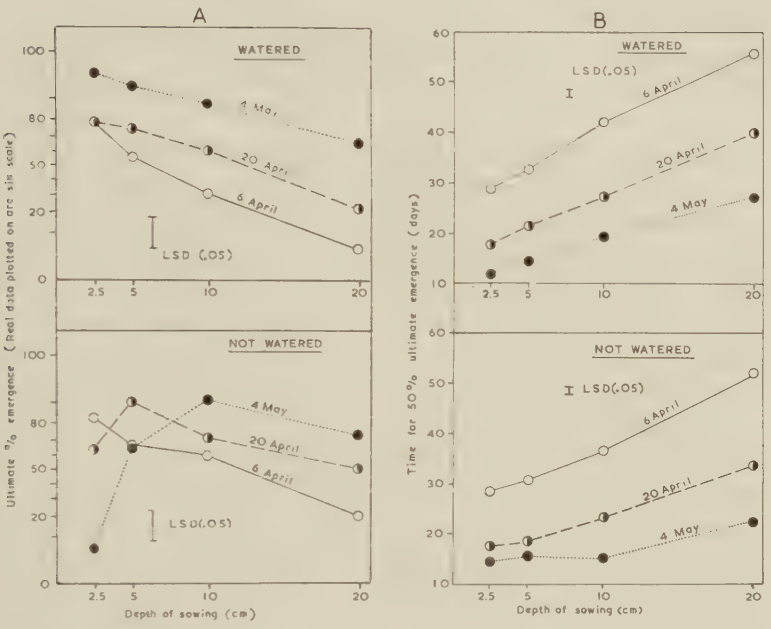

Fig. 3. The influence of depth of sowing on A. *Ultimate % emergence* (arc sin transformed data) and B. *Time for 50% ultimate emergence* (days), of White Horsetooth maize sown at three sowing dates in watered and unwatered field plots (1956).

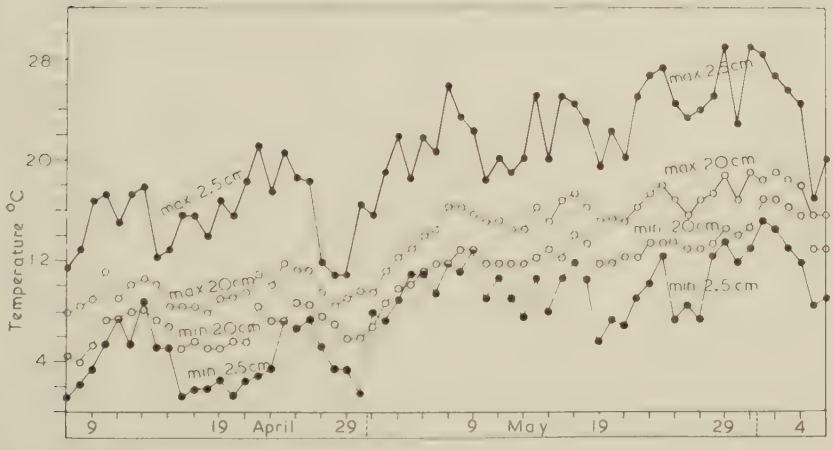

Fig. 4. Daily maximum and minimum soil temperatures in the field at 25, and 20 cm depth (data for 1956).

ment of seedlings was from grains sown at 2.5 cm on April 6th, at 5 cm on April 20th and at 10 cm on May 4th; this is in contrast with the watered plots from which the best emergence was always from 2.5 cm depth.

The temperatures of the watered plots remained at or slightly below that of the control plots and the difference between them was most marked during dry periods, presumably due to changes in the thermal capacity and conductivity of the soil and evaporation following watering. In this respect it is significant that watering did not increase *ultimate percentage emergence* from 10 or 20 cm depth from any sowing date – in fact watering reduced emergence from those depths and increased the *time for 50 per cent emergence*.

DISCUSSION

The experiments described above demonstrate that considerable mortality may result in a maize sample from sowing too deep and that there is a general inverse relationship between the *ultimate percentage emergence* and the *time for 50 per cent emergence* provided that moisture is not limiting.

A simple relationship between depth of sowing, *time for 50 per cent emergence* and *ultimate percentage emergence* is obtained from experiments when the temperature is controlled and moisture level is maintained near field capacity. However such a simple relationship is upset in the field in the following ways:

(1) Drying out of the surface layers of the soil may lower the zone from which maximal emergence occurs so that from different dates of sowing in the field maximal emergence may occur from 2.5, 5, or 10 cm. When plots are regularly watered most rapid and successful emergence is always from 2.5 cm. It is important to remember that grains which are prevented from germinating because the soil is too dry or too cold, are exposed to damage from pathogenic attack (Harper [7 8]).

(2) Emergence is reduced when the period following sowing is cold, and deep sowing exaggerates this effect.

(3) Differences between pot and field experiments are emphasised by the greater extremes of temperature in the pots, although the differences in soil temperature between depths are minimised in pots. This reveals an important characteristic of pot culture which is frequently ignored.

(4) Bird damage was not significant in these experiments but it is general field experience that shallow grown grain is more frequently taken by rooks and other birds.

The choice of a suitable sowing depth for maize must therefore be determined by the following considerations: –

(a) Shallow sowing at 2.5 cm is optimal if the seed bed is wet enough to allow germination or if weather forecasts predict rainfall within a few days of sowing. However, precautions must then be taken to scare birds.

(b) If the soil is dry at sowing time and there is no forecast of likely rain, sowings at 5 or even 10 cm depth may be desirable for successful seedling establishment: they also reduce bird damage.

(c) Very deep sowing – even as deep as 20 cm – may give field stands as high as 70–80 per cent of the sown grains particularly from late sowings. It must, however, be emphasised that this finding is for a large-grained variety, White Horsetooth, and that varieties with smaller grains may not withstand such deep sowing successfully.

SUMMARY

Maize (variety White Horsetooth) was sown at depths of 2.5, 5, 7.5 and 10 cm in pot and field experiments and at 2.5, 5, 10 and 20 cm in field experiments. *Ultimate % emergence* and *time for 50% emergence* were determined, and soil temperature measurements were made. Emergence was reduced at all depths greater than 2.5 cm when soil moisture conditions were maintained near field capacity. However the optimal depth of sowings in the field may be 5 or even 10 cm during periods of drought. The relationships between soil temperature and depth are compared for field and pot conditions and the dangers of extrapolating from the pot to the field are emphasised. The factors which should be considered in deciding the depth at which maize should best be sown are discussed.

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THE CONVERSION OF AMINO ACIDS IN SOILS *

I. AMINO-ACID BREAKDOWN AND NITRIFICATION IN CULTIVATED AND NATURAL SOILS

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INTRODUCTION

It is now well established that the breakdown of organic compounds of nitrogen in soils involves a series of changes promoted by different types of soil micro-organisms. The terms ammonification and nitrification have been widely applied to these processes, ammonification comprising the decomposition of organic nitrogenous compounds to ammonia, and nitrification the oxidation of ammonia to nitrite and nitrate. Under favourable conditions, the sequence of changes from ammonia to nitrite and nitrate can be observed, when soils are percolated with soluble nitrogenous compounds, such as amino acids [12].

The soil processes of ammonification and nitrification are sharply contrasted in several ways, but particularly with regard to the effect of organic matter on the micro-organisms associated with them. Ammonifying organisms are predominately heterotrophic; their proliferation and activity in the soil depends on a readily available supply of organic matter. The nitrifying organisms, on the other hand, are autotrophic and organic compounds, particularly amino acids, are often toxic to these organisms, at least in pure culture [6]. It would appear, therefore, that conditions favourable to ammonifying organisms are in some respects unsuitable for those which promote nitrification.

There are, however, several other ways in which the active

* These studies comprise part of the data submitted in a Ph. D. thesis to the University of Bristol in 1953.

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breakdown of organic matter in the soil may interact with nitrification. The proliferation of heterotrophic micro-organisms is generally associated with the assimilation of suitable nitrogenous nutrients and, as shown by Lees [7], temporary immobilisation of nitrate may occur in this way. A further possibility is the loss of nitrogen by denitrification. There is now strong evidence from the work of Corbet and Wooldridge [4] and Meiklejohn [9] that active denitrification may take place during the breakdown of organic matter under aerobic conditions.

The primary aim of the experiments described in this paper was to obtain further information concerning the interaction between ammonification and nitrification processess in the soil. With this object, the breakdown of amino acids to ammonia and its effect on subsequent nitrification has been investigated in a range of soils by means of a percolation method similar to that developed by Lees and Quastel [8].

EXPERIMENTAL METHODS

1. Soils and their collection

During the course of this investigation, soils were collected from nine localities in the neighbourhood of Bristol; two of these were from cultivated gardens and the others from natural or semi-natural grassland and woodland sites, as summarised in Table I.

TABLE I

Soils used in percolation experiments

Station	Type of vegetation	pH	Free carbonate (HCl test)
Hiatt Baker Garden (Univ. of Bristol)	Cultivated garden	7.0	trace
Long Ashton	,, ,,	6.0	nil
Brislington	Grassland pasture	7.8	strong
Savernake Forest	Beechwood	6.5	trace
Marlborough Downs I	Chalk grassland	8.0	strong
,, ,, II	Mixed deciduous wood	6.0	nil
Lord's Wood, Pensford, I	Oakwood	5.3	nil
,, ,, ,, II	Mixed deciduous wood	6.4	trace
,, ,, ,, III	Birch wood	7.7	moderate

The most extensive studies of both ammonification and nitrification were carried out with Brislington and Long Ashton soils, the others being chosen to give a fairly wide range of calcareous and non-calcareous soils of the

district. Each soil was collected from the main rooting zone, usually 3–6 inches from the surface, and brought to the laboratory in a sterilized glass jar, care being taken to avoid handling. The soil samples were then shaken in a sterilised tier of sieves, the fraction between 2 4 mm being collected and weighed out into the soil tube of the percolation apparatus.

The dry weight of the soil used in the percolation units was estimated by drying weighed samples at 105°C for 24 hours and then reweighing.

2. *Percolation technique*

The breakdown of the amino acids was studied by means of percolating units similar to those described by Audus [1]. Percolation was operated by air pressure from a compressed-air supply connected to the sampling tubes of the units. The pressure was stabilised by means of a mercury escape valve. The airstream was first freed from carbon dioxide by passing through soda-lime towers and from ammonia by bubbling through dilute acid, and then distributed to a series of units by a glass manifold, each lead carrying a small piece of capillary tubing to equalize the airflow. It was possible to use a maximum of 25 g of soil and 170 ml of percolate in each unit. The ratio of air to liquid passing over the soil was approximately 4 : 1 and the volume of air passing through the soil in a unit approximately 4 litres per hour. For all experiments, the units were set up in a dark room (to prevent algal growth on the soil) at 19 $\pm$ 1°C. Fresh, undried soil was used for most of the experiments, since in a preliminary test it was shown by plate counts that drying caused a marked reduction in the number of heterotrophic micro-organisms. Ammonification undoubtedly involves many organisms of this type, and drying was therefore considered undesirable. To facilitate the percolation of fresh soil a fairly coarse soil fraction (2-4 mm) was used and the amounts generally limited to 10 g. By using a narrow-bore lift tube (2 mm) it was possible to obtain satisfactory percolation with a comparatively slow airstream; this was also advantageous with fresh soils.

Where amino acids were used, the required amount of soil was inserted into the soil tube by means of a sterile spatula and the unit assembled under aseptic conditions.

3. *Chemical analyses*

Changes brought about in the soil were followed by means of chemical analyses of the percolate. Analyses were carried out for amino, ammonium, nitrite and nitrate nitrogen. Some estimations were also made of the total nitrogen content of both soils and percolates.

Amino-N. Amino-N was estimated using a method similar to that described by Woiwod [16]. The amino-acid solution (containing not more than 50 μg NH_2-N per sample) was shaken with a copper phosphate suspension at pH 9.0. Excess copper phosphate was removed by centrifuging and the copper in the soluble complex was estimated colorimetrically as diethyldithiocarbamate, using a Spekker absorptiometer with violet filters (Ilford no. 601) and 1-cm cells. The amount of amino-N corresponding to the ab-

sorptiometer readings was obtained from a calibration curve based on a series of estimations with standard solutions of the appropriate amino acid. The method used here differed from that given by Woiwod in that the excess copper phosphate was removed by centrifuging not by filtration, since it was found that some batches of filter paper gave irregular results. Centrifuging for 25 minutes at 2,000 rpm gave satisfactory and reproducible values.

Ammonium-N. Ammonium-N was estimated by the boric acid-HCl micro-diffusion method of Conway [3], using a no. 1 size Conway unit (A. G. Gallenkamp & Co.). Titrations were carried out with $N/28$ HCl in an "Agla" microsyringe mounted as a burette [14]. With solutions of pure ammonium, sulphate, the method was found to give values accurate within $\pm$ 1% over a range 100-20 μg NH_4–N per 0.5-ml sample and within $\pm$ 2% down to 10 μg.

Nitrite-N. Nitrite-N was estimated colorimetrically by means of the Griess-Ilosvay reagents described by Rider and Mellon [13]. The colorimetric readings were carried out on a Spekker absorptiometer using green filters (Ilford no. 604) and 2-cm cells. With pure solutions of nitrite the method was found to be accurate within $\pm$ 0.05 μg nitrite-N.

Nitrate-N. Nitrate-N was estimated indirectly, using the microdiffusion method of Conway [3]. Nitrate was first reduced to ammonia which was then estimated as described above, the whole reaction being carried out in the microdiffusion unit. The titration figure gave the total amount of nitrate-, nitrite- and ammonium-N in the percolate sample. Separate estimations of nitrite- and ammonium-N were carried out at the same time and the nitrate value obtained by difference. Tests with standard solutions of sodium nitrate indicated that an accuracy of $\pm$1% could be achieved over a range 10–100 μg N per 0.05-ml sample. Analyses of known mixtures of sodium nitrate and ammonium sulphate showed that the recovery of total inorganic nitrogen was generally within 1%. Ammonium-N recovery tended to be slightly greater than 100% (101–102%) and nitrate-N recovery slightly lower (95–99%). There was good agreement between estimations of nitrite by the micro-diffusion method and by the colorimetric method. For quantities of nitrite-N below 20 μg/ml estimation by the microdiffusion method was less reliable, but satisfactory analyses could be carried out colorimetrically with nitrite-N concentrations as low as 0.5 μg N per ml.

Total nitrogen. The estimates of total-N were made by the Kjeldahl method described by Chibnall, Rees and Williams [2]. Where necessary, a preliminary reduction of nitrite and nitrate was effected by means of treatment with reduced iron as recommended by Pucher, Leavenworth and Vickery [11]. Ammonia was determined in a suitable aliquot of the steam distillate by treating with Nessler's solution [15] and estimating colorimetrically using a Spekker absorptiometer with violet filters (Ilford no. 601) and 2-cm cells.

4. Measurements of carbon dioxide output and oxygen uptake of soils

Carbon dioxide output. The production of carbon dioxide during percolation of the soil was measured by means of Pettenkoffer tubes filled with barium hydroxide, connected to the outlet tubes of the percolation units. 25 ml *N*/20 barium hydroxide were run into each tube and the tube filled with CO_2-free water. The airstream was adjusted to give a steady bubble rate through the tube (about 40 bubbles per minute). After a suitable period, usually 24 hours, the contents of each Pettenkoffer tube were titrated against *N*/20 HCl with phenolphthalein as indicator.

Oxygen uptake. Oxygen uptake of fresh or percolated soils was measured manometrically in a Warburg apparatus. The technique was similar to the direct method described by Dixon [5].

5. pH measurements

pH measurements were carried out by means of either B.D.H. indicators or a glass electrode standardised against acid-phthalate or borate buffers.

6. Plate counts of soil bacteria

In some experiments, the number of heterothrophic bacteria in percolated soil was estimated by a plate-count method. Suitable dilutions of a soil sample (1/1,000,000; 1/10,000,000) were prepared using sterile water. 1-ml aliquots of these dilutions were used per petri plate. The medium used was a sodium glutamate agar, viz.:

K_2HPO_4	1.0 g
$CaCl_2.6H_2O$	0.1 g
NaCl	0.1 g
$MgSO_4.6H_2O$	0.3 g
$FeCl_3$	trace
Glutamic acid	0.68 g
Agar	15.0 g
Distilled water	1 litre

The basic constituents were dissolved in 900 ml distilled water, distributed in 90-ml portions into small flasks and sterilised by autoclaving. The glutamic acid was dissolved in a small volume of distilled water, the pH adjusted to 8.0 with sodium hydroxide and the volume made up to 100 ml. This was then sterilised by filtration. Before pouring plates, 10 ml of the glutamate solution was added with a sterile pipette to each flask of basic medium, cooled to 45°C.

The soil plates were incubated at 20°C for one week and the number of bacterial colonies per plate then counted.

EXPERIMENTAL RESULTS

1. The breakdown of amino acids and ammonification

Course of ammonification. All the experiments in which

fresh soils have been percolated with dilute solutions of amino acids such as glycine and glutamic acid have shown that there is a rapid loss of amino-N from the percolate associated with an increase of free ammonia. The mean results of a typical experiment in which six samples of Brislington soil were treated with glutamate are shown in Fig. 1., and similar data obtained when glycine was used are shown in Fig. 2.

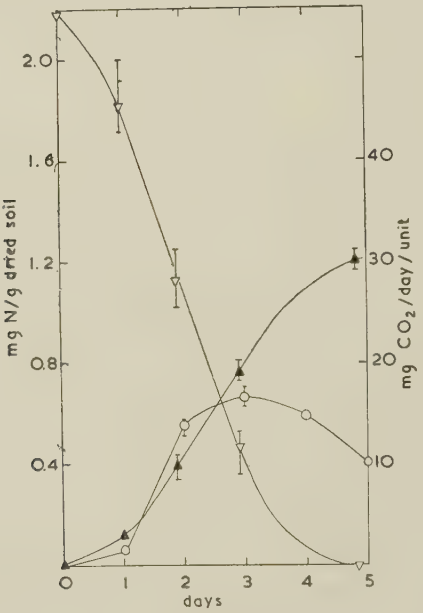

Fig. 1. Ammonification in six replicate samples of 10 g Brislington soil percolated with $M/140$ glutamate. Mean results are shown and variation between different samples indicated by vertical lines.
Symbols: Amino-N ▽, Ammonium-N ▲
Rate of CO_2 production -◯-

In these and other experiments there was clear evidence that the amino acid was rapidly utilised; after a preliminary lag phase of about 24 hours the production of ammonia followed a roughly sigmoid curve reaching a maximum after 3–8 days in the different soils which were studied. During ammonification, there was a sharp rise in the rate of carbon-dioxide loss from the soil indicating an extensive breakdown of the carbon skeleton of the amino acid. In comparable samples of the same soil the increase of carbon-

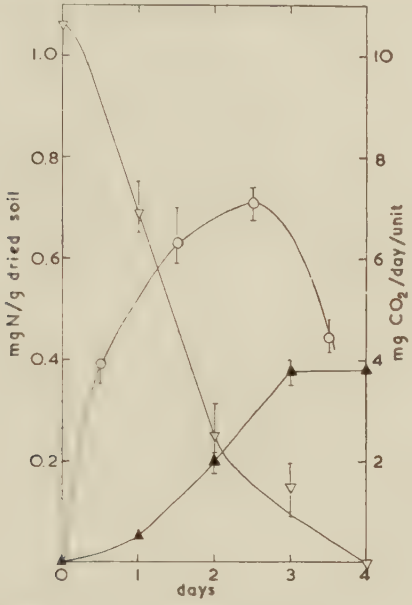

Fig. 2. Ammonification in six replicate samples of 10 g Brislington soil percolated with *M*/280 glycine. Mean results are shown and variation between samples indicated by vertical lines.
Symbols: Amino-N ▽, Ammonium-N ▲
Rate of CO_2 production -○-

dioxide production with glutamic acid was much greater than with glycine, probably owing to the greater amount of carbon supplied and higher C N ratio of glutamic acid. Calculations show that in the different soils 28 50% of the carbon supplied in the amino acid may be accounted for by the increase of carbon-dioxide production, the remainder presumably being assimilated by the soil micro-organisms.

It is of interest that the course of ammonification observed in many of these experiments does not conform with the equations for autocatalytic reactions developed by Miyake [10], Lees and Quastel [8] and Quastel and Scholefield [12], from their studies of ammonification and nitrification. In general, the amounts of ammonia recovered during the early stages of percolation are lower than expected for an autocatalytic reaction and it seems probable that base exchange and assimilation of nitrogen by the soil micro-organisms may be mainly responsible for this.

Yield of ammonia. A consistent feature of the experiments was that after completion of ammonification only a part of the amino-N supplied to the soil was recovered as free ammonia in the percolate. The remainder of the nitrogen was held in some way in the soil and percolate and the term immobilisation has generally been applied to this phenomenon. The extent of this immobilisation in a range of soils percolated under similar conditions with $M/140$ glutamate is shown in Table II.

TABLE II

Immobilisation of nitrogen during glumatic acid breakdown in a range of soils

Soil	% N	
	As NH_4^+ in percolate	Immobilised
Hiatt Baker garden	48.8	51.1
Long Ashton	53.5	46.5
Brislington	53.1	46.9
Marlborough Downs I	31.8	69.2
,, ,, II	48.8	51.2
Savernake Forest	34.8	65.3
Lord's Wood, Pensford I	47.6	52.4
,, ,, ,, II	40.6	59.4
,, ,, ,, III	40.6	59.4

In none of these experiments was appreciable nitrification detected during the ammonification of glutamate, so that the low recovery of ammonium-N, varying from 31–53% in different soils, cannot be attributed to the production of nitrite or nitrate. Judged by the amount of ammonia released relative to the disappearance of amino-N, immobilisation appears to be most rapid during the early stages of percolation with the amino acid. As already noted, it is probable that base exchange in the soil together with assimilation of nitrogen by micro-organisms are mainly responsible for the low yields of ammonia.

Proliferation of ammonifying organisms in the soil. Direct evidence of a rapid increase in the number of heterotrophic micro-organisms in soils during the early stages of percolation has been obtained by means of plate counts. The results of an experiment in which 10 g Brislington soil were percolated with $M/140$ glutamate and samples of soil removed at intervals for plating-out

are shown in Table III. Over a period of 24 hours, nearly a seventy-fold increase of heterotrophic micro-organisms was observed.

TABLE III

Proliferation of bacteria during percolation of Brislington soil with glutamate

Hours	μg NH_4–N formed per ml percolate	Millions of bacteria per g soil
0	0	7.7
12	0	28.2
24	6.5	490
48	20.06	>500

The high rates of ammonification, following the initial lag period, may therefore be attributed to the proliferation of micro-organisms in the soil. This has been confirmed in many experiments in which soils, after a preliminary percolation with an amino acid and completion of ammonification, have been percolated a second time with a solution containing a similar concentration of the amino acid. Typical data showing the course of ammonification during the first and second percolations of Hiatt Baker soil with glutamate are given in Fig. 3. During the second percolation the loss of amino-N and release of ammonia followed an approximately linear course, the lag phase having been eliminated. There is little doubt that the increased rate of ammonification was a result of the proliferation of micro-organisms and corresponded closely to the "enriched" or "bacteria-saturated" soils, as discussed by Lees and Quastel [8] in connexion with nitrification processes.

Some further evidence of increased activity of micro-organisms in the soil after percolation with amino acids has been obtained by measurements of oxygen uptake. In experiments with Brislington soil, it was shown that after percolating the soil with glutamate the rate of oxygen uptake, both with or without additional glutamate, was much greater than that of comparable samples of fresh soil. For these experiments 25 g of the soil were first percolated for 36 hours with 150 ml of $M/140$ glutamate. This was then washed through with distilled water and allowed to drain for 1–2 hours to remove excess moisture. Six samples each, of 2 g, were then weighed into Warburg flasks; three of them were treated with 0.5 ml of approximately $M/3$ glutamate and three with 0.5 ml distilled water.

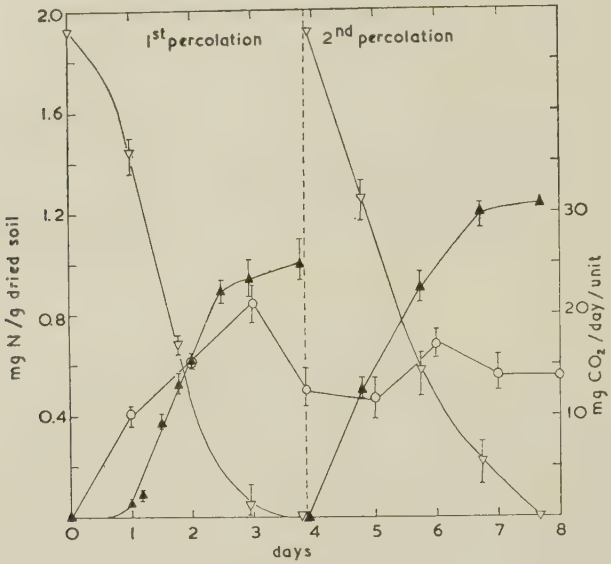

Fig. 3. Ammonification in six replicate samples of 10 g Hiatt Baker soil during two consecutive percolations with $M/140$ glutamate. Mean results are shown and variation between samples indicated by vertical lines.
Symbols: Amino-N ▽, Ammonium-N ▲
Rate of CO_2 production -◯-

Measurements of oxygen uptaken were made in a similar way with samples of freshly collected soils. The mean results are shown together in Fig. 4.

The rates of oxygen uptake in the different samples were nearly constant over the period of two hours in which measurements were taken. The Q_{0_2} of the soil subjected to percolation with glutamate was about double that of the fresh soil. In both soils, the addition of glutamate led to more than a twofold increase in oxygen uptake, although the absolute increase was very much greater in pretreated soil. Since the Q_{0_2} of the soil may be regarded as a general measure of the activity of the soil micro-organisms, it may be deduced that the percolated soil had become enriched with micro-organisms capable of utilising the amino acid.

Although the data described above indicate that the soil crumbs become enriched with bacteria during ammonification, the possibility of proliferation of micro-organisms in the percolating solution cannot be eliminated. A test of the relative importance of the

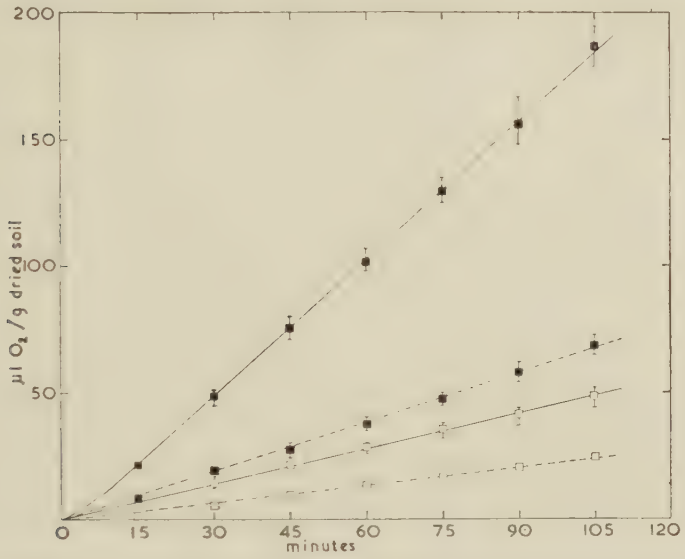

Fig. 4. Oxygen uptake of fresh and percolated soil with and without the addition of glutamate

Symbols: percolated (enriched) soil ——————
fresh soil – – – – – –
with glutamic acid ■
with distilled water □

activity in soil and percolate was made by an experiment in which the effects of removal of the soil were investigated.

Three units were set up with 10 g Brislington soil and 150 ml *M* 140 glutamate. After about 2½ days the soil was removed from one unit and the unit operated with percolate alone. The soil thus removed was placed in a new unit and repercolated with a fresh solution of glutamic acid. Two units were retained as controls. The results are presented graphically in Fig. 5. When the soil was removed, amino-acid utilisation and ammonia formation almost ceased, and carbon-dioxide measurements showed a sudden fall. The soil that was removed gave a nearly linear rate of ammonium-N formation, indicating that it had become enriched with the effective micro-organisms. It is highly probable, therefore, that under the conditions of percolation used here, the majority of ammonification took place in the soil rather than in the percolate.

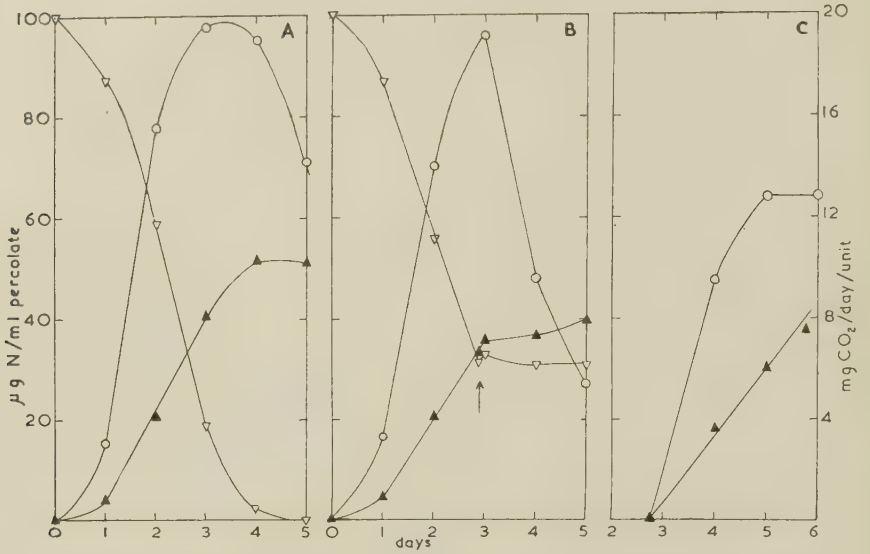

Fig. 5. Effect of the removal of soil during ammonification in Brislington soil.

A. Percolation of soil with $M/140$ glutamate.

B. Similar to A, except at point indicated by arrow soil removed and percolation continued with percolate alone.

C. Soil transferred from B to new unit and repercolated with glutamate.

Symbols: Amino-N ▽, Ammonium-N ▲

Rate of CO_2 production -◯-

2. Nitrification of ammonia

The ability of the different soils to promote nitrification was studied by percolating them with dilute solutions of ammonium sulphate. With three of the soils investigated active nitrification was observed under these conditions and the course of nitrification and enrichment with nitrifiers was similar to that already described for other soils by Lees and Quastel [8]. The results of a typical experiment with Hiatt Baker soil are given in Fig. 6.

The ammonium-N content of the percolate showed a marked fall at the beginning of percolation. This represented the ammonia retained in the soil by base exchange.

The amount of nitrite accumulating in the percolate, both with Hiatt Baker and Brislington soil was appreciable, reaching a maximum figure of approximately 300 μg per g dried soil. This contrasts with the results of Lees and Quastel on Rothamsted

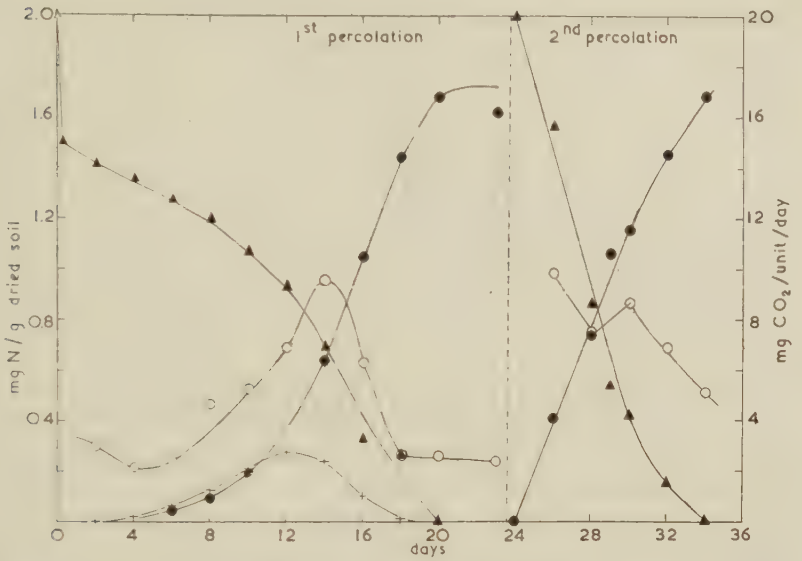

Fig. 6. Nitrification in six replicate samples of 10 g Hiatt Baker soil during two successive percolations with $M/280$ ammonium sulphate. Mean results are shown.

Symbols: Ammonium-N ▲, Nitrite-N +
Nitrate-N ●
Rate of CO_2 production -◯-

soils [8] where only traces of nitrite were observed during nitrification. There were indications that the accumulation of nitrite was related to the amount of carbon dioxide in the airstream. When a small amount (approximately 5%) of carbon dioxide was introduced into the airstream during the nitrification of ammonium sulphate in Brislington soil, much smaller quantities of nitrite were detected throughout the course of the experiment (Table IV). In most of the experiments, the initial level of carbon dioxide was very low,

TABLE IV

Effect of carbon dioxide on nitrite formation in Brislington soil

Quantities in μg NO_2–N per ml percolate

Day no.	1	3	5	6	8	10	11	13	15
+ 5% CO_2 in airstream	0	0	0	0	0	0.4	0.4	0.6	0.9
CO_2-free	0	0	0	5.6	9.7	18.9	22.3	12.2	0

due to the passage of the incoming airstream through soda-lime. This may have limited the rate of conversion of nitrite to nitrate and thus resulted in an accumulation of nitrite.

It is significant that rapid conversion of nitrite to nitrate during nitrification in Hiatt Baker soil followed a rise in carbon-dioxide output, probably due to liberation of carbon dioxide from soil carbonates and the percolate by the nitrous acid formed. In the experiments of Lees and Quastel [8], no removal of carbon dioxide from the airstream was carried out and apparently the level was sufficient to allow the conversion of nitrite to nitrate to proceed rapidly.

By comparing the rates of nitrate formation from ammonium sulphate and from sodium nitrite in Brislington soil, further evidence was obtained that under the experimental conditions employed here the conversion of nitrite to nitrate was a limiting reaction. Three 10-g samples were percolated with 150 ml $M/560$ ammonium sulphate and three with 150 ml $M/280$ sodium nitrite. The result is shown in Fig. 7. The rate of nitrate formation was similar in both sets of soils. Nitrite accumulated during the conversion of ammonium sulphate to nitrate, indicating that the nitrite → nitrate reaction was limiting the complete nitrification process.

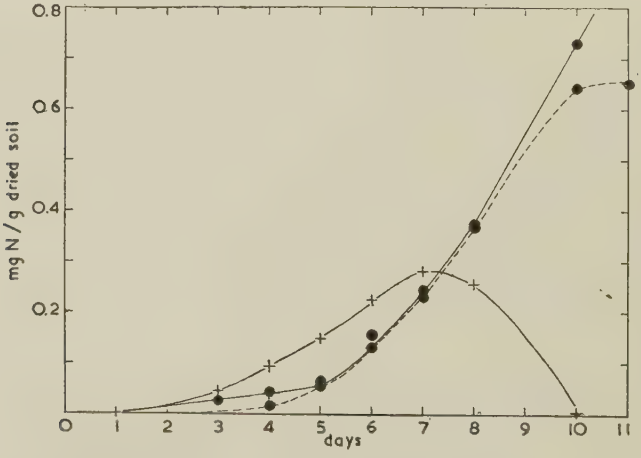

Fig. 7. Conversion of ammonium sulphate and sodium nitrite to nitrate in three replicate samples of 10 g Brislington soil. Mean results are shown.
Symbols: Soil percolated with ammonium sulphate ————————
Soil percolated with sodium nitrite – – – – – –
Nitrite-N +, Nitrate-N ●

Of the nine soils investigated, five showed little or no nitrification even after prolonged percolation with ammonium sulphate (Table V).

TABLE V

Nitrification of ammonia in a range of soils.
10g of each soil (4—2 mm) percolated with 150 ml
M/280 ammonium sulphate

Soil	Lenght of expt. (days)	% Nitrification
Hiatt Baker garden	22	90
Long Ashton	12	10
Brislington	22	88
Marlborough Downs I	12	none
„ „ II	12	none
Savernake Forest	12	none
Lords's Wood, Pensford I	12	none
„ „ „ II	12	none
„ „ „ III	24	94

The reasons for this failure to promote nitrification have not been fully investigated, but addition of calcium carbonate to Long Ashton soil resulted in a marked increase in nitrification, which suggests that the pH may be unfavourable for nitrification in this soil.

In those soils which did promote the nitrification of ammonium sulphate, 88-90 per cent of the nitrogen added as ammonium-N was ultimately recovered as nitrate, although the initial fall in ammonium-N concentration due to base exchange was frequently 20 per cent. Most of the nitrogen added was therefore recovered, and assimilation by micro-organisms may well account for the small deficit observed.

3. Nitrification following ammonification

Ammonium-N produced by the breakdown of an amino acid in soil becomes available for nitrification by the nitrifying bacteria. The aspects of amino-acid conversion which are considered here are its role in supplying material for nitrification and the indirect effects of the amino acids (and their decomposition products) on this process. The problem was first studied by percolating fresh soil with the amino acid and then following the course of ammonification

and nitrification. This is a sequence of changes which might be expected to occur naturally in soils.

Of the three soils generally used in the present investigation, Hiatt Baker and Brislington soils nitrify well with ammonium sulphate; Long Ashton soil does not. In contrast to the poor rate of nitrification with ammonium sulphate, ammonia produced from glutamic acid was fairly rapidly converted to nitrate in Long Ashton soil: after ten days' percolation, 75 per cent of the amino-N had been recovered as nitrate-N. In a comparable experiment with ammonium sulphate, only 10 per cent of the ammonium-N was converted to nitrate-N in a similar period of time. The course of ammonification followed by nitrification in this soil is shown in Fig. 8.

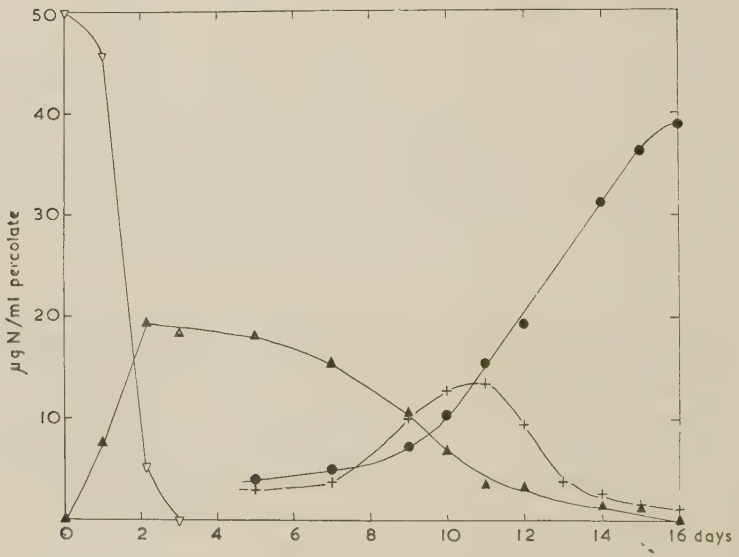

Fig. 8. Conversion of glutamic acid to nitrate in three replicate samples of 10 g Long Ashton soil. Mean results are shown.
Symbols: Amino-N ▽, Ammonium-N ▲
Nitrite-N +, Nitrate-N ●

It is probable that the improvement in nitrification is due in part to a favourable change in pH. Quastel and Scholefield [12] have shown that in several slightly acid soils there is a more rapid production of nitrate from amino acids than from ammonium

sulphate. They attribute this to the fact that less acid is produced from an amino acid during nitrification than from an ammonium salt. As already noted, nitrification in Long Ashton soil can be increased by the addition of calcium carbonate.

The overall yield of nitrate after percolation of Long Ashton soil with glutamate amounted to 70–75 per cent of the nitrogen added and it is clear that this value is appreciably higher than the yield of ammonium-N from ammonification in this soil (see Table II). The higher yield of nitrate may be largely attributed to the recovery of ammonium-N held by base exchange in the soil. Under comparable conditions it was found that during the percolation with ammonium sulphate about 20 per cent of the ammonia added was held by base exchange in the Long Ashton soil.

Similar experiments were carried out with Brislington and Hiatt Baker soils, and typical data are shown in Fig. 9. Ammonification followed by nitrification took a course similar to that described above, but the recovery of nitrate, when all the ammonia had been utilised, was appreciably lower than the comparable value for Long Ashton soil.

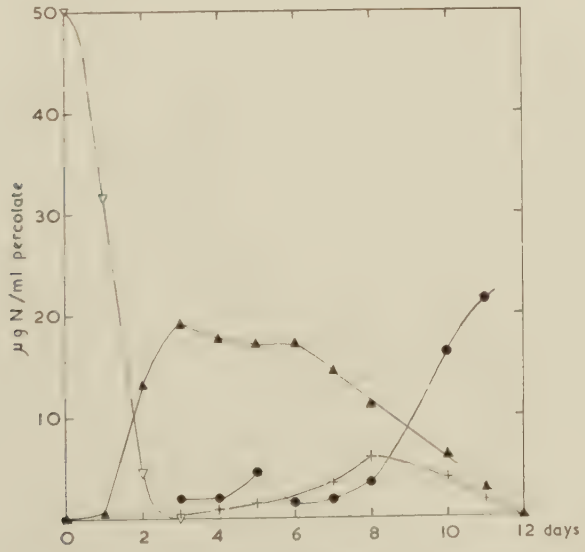

Fig. 9. Conversion of glutamic acid to nitrate in three replicate samples of 10 g Brislington soil. Mean results are shown.
Symbols: Amino-N ▽, Ammonium-N ▲
Nitrite-N +, Nitrate-N ●

The conversion of glycine to nitrate during the percolation of Brislington soil is shown in Fig. 10. Compared with glutamate, glycine promoted more rapid nitrification and there was a higher yield of nitrate, amounting to 66 per cent of the amino-N added, compared with a 47 per cent recovery from glutamate.

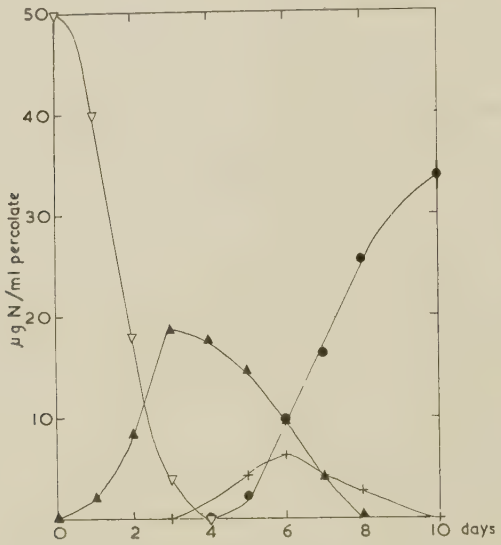

Fig. 10. Conversion of glycine to nitrate in six replicate samples of 10 g Brislington soil. Mean results are shown.
Symbols: Amino-N ▽, Ammonium-N ▲
Nitrite-N +, Nitrate-N ●

In a further experiment with this soil, a direct comparison was made of nitrification in replicate samples of fresh soil percolated with dilute solutions of ammonium sulphate, glycine and glutamate containing equal amounts of nitrogen. The results (Fig. 11) show that nitrification from glutamate was much slower than from ammonium sulphate, glycine occupying an intermediate position. In particular, the nitrification curve for glutamate showed a pronounced lag phase of 4–6 days and the yield of nitrate, after all the ammonium-N had been taken up from the percolate, amounted to only 47 per cent of the amino-N added, as against 69 per cent for glycine and 88 per cent for ammonium sulphate. Under comparable conditions, the mean recovery of ammonia from glutamate in six

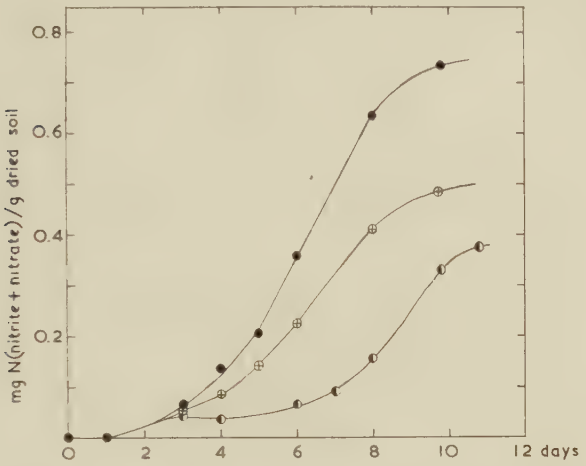

Fig. 11. Comparison of the rates of conversion of ammonium sulphate, glycine and glutamic acid to nitrate in Brislington soil. Mean results of three replicate units given for each nitrogen source.

Symbols: Soil percolated with *M*/560 ammonium sulphate ●
Soil percolated with *M*/280 glycine ⊕
Soil percolated with *M*/280 glutamic acid ◐

replicate samples of this soil was 54 per cent of the amino-N added. Evidence given in Section 1 indicates that a further 20–25 per cent of the nitrogen is held by this soil as ammonia in base exchange. After nitrification, therefore, a higher yield of nitrate would be expected than the 47 per cent actually obtained. The reduced yield of nitrate from glutamate may be attributed, at least in part, to greater immobilisation of nitrogen through assimilation by micro-organisms in the soil. The possibility that it may also arise as a result of the interaction between different components of the soil microflora will be considered more fully in a later section.

The amount of the amino acid used in the preliminary percolation of the soil has an important effect on the subsequent nitrification process. With Brislington and Hiatt Baker soils much larger amounts of nitrite accumulated when the soils were subjected to more prolonged percolation with larger amounts of the amino acid. The results of two experiments in which the fresh soil was percolated twice with glutamate before nitrification was allowed to proceed are given in Fig. 12. In these experiments the soil was

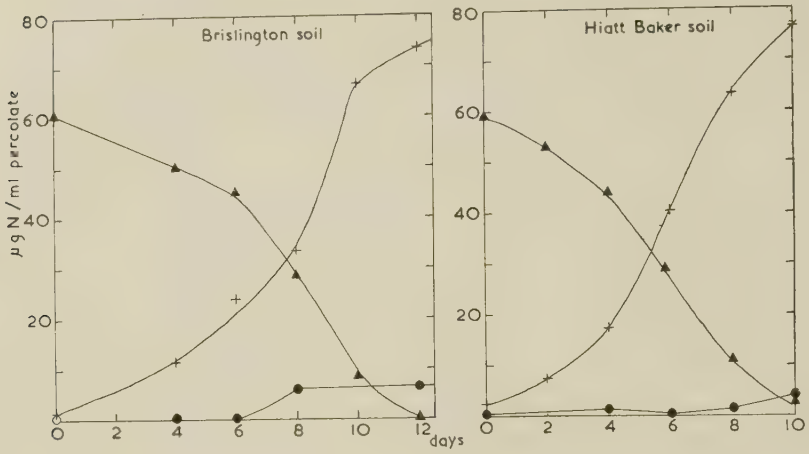

Fig. 12. Nitrification in Brislington and Hiatt Baker soils following two percolations with glutamate. For each soil mean results from six replicate samples of 10 g are shown.

Previous treatment of soil: percolation with $M/280$ glutamate followed by percolation with $M/140$ glutamate until ammonification complete.

Symbols: Ammonium-N ▲, Nitrite-N +
Nitrate-N ●

first percolated with 150 ml $M/280$ glutamate and, when ammonification was complete, it was washed through with distilled water and repercolated with the same volume of $M/140$ glutamate. Following this treatment with the amino acid, there was a very marked accumulation of nitrite, so that when the ammonia in the percolate was very nearly exhausted 90–94 per cent of the products of nitrification were in the form of nitrite, with relatively small amounts of nitrate. The relative rates of nitrite and nitrate formation are clearly very different from those obtained when the soil was subjected to a single percolation with the amino acid (cf Fig. 9).

4. Ammonification and nitrification in enriched soils

It might be expected that a soil which has been percolated with an amino acid and allowed to nitrify, as described in the preceding section, would be enriched with both the heterotrophic and the autotrophic micro-organisms which promote ammonification and nitrification. Direct evidence of this double enrichment has been

obtained in several experiments with soils percolated with glycine. For example, an experiment was carried out in which six replicate samples of Brislington soil were percolated with *M* 280 glycine for ten days until all the ammonia had been converted to nitrate. Three of the samples were then treated with *M* 280 ammonium sulphate and the other three with *M* 140 glycine. The results of this second percolation are shown in Fig. 13. The nitrification of

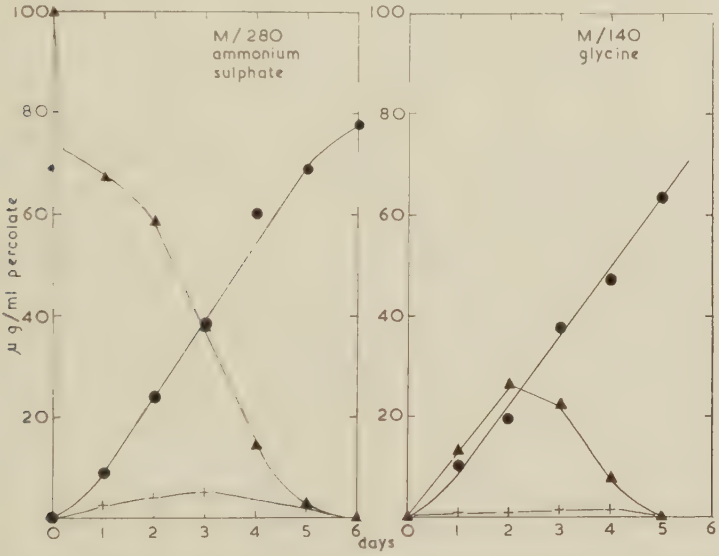

Fig. 13. Conversion of ammonium sulphate and glycine in enriched Brislington soil. For each treatment, mean results from three replicate samples of 10 g are shown.
Previous treatment of soil: percolation with *M*/280 glycine until nitrification complete.
Symbols: Ammonium-N ▲, Nitrite-N +
Nitrate-N ●

ammonia proceeded at a high and almost linear rate, indicating that the soil was enriched with nitrifiers. With glycine, a high and approximately linear rate of nitrate formation was also obtained similar to that with ammonium sulphate, the absence of an appreciable lag phase indicating the dual enrichment of the soil with ammonifiers and nitrifiers.

Under some conditions, however, it was found that after pretreatment with an amino acid, nitrification was very much de-

creased and this resulted in a reduced yield of nitrate. The failure of nitrification was especially marked in soils which had previously been subjected to relatively prolonged percolation with glutamate, as described on p. 68, Fig. 12. These soils, as already shown, had a marked tendency to form large quantities of nitrite, possibly owing to the effect of the pretreatment with glutamate on the proliferation or activity of the nitrate-forming bacteria. Experiments with both Hiatt Baker and Brislington soils brought into that condition, were carried out as follows. Six samples of fresh soil were percolated with 150 ml $M/280$ glutamate until ammonification was complete, washed through with distilled water and then repercolated with $M/140$ glutamate for five days. After this time, most of the ammonia had been converted to nitrite. Three of the six replicate samples were then repercolated with $M/140$ glutamate and the other three with $M/280$ ammonium sulphate. The results of analyses carried out during the percolation of Brislington soil treated in this way are shown in Fig. 14.

With ammonium sulphate, nitrification proceeded rapidly. For the first three days mainly nitrite was produced but after five days the recovery of nitrogen from the ammonium sulphate was 87 per cent consisting of approximately equal quantities of nitrite and nitrate. By comparison, in soils percolated with glutamate, nitrification was markedly reduced. Small amounts of nitrite could be detected in the percolate followed later by traces of nitrate. At the end of five days only 26 per cent of the nitrogen added could be recovered either as ammonia-, nitrite- or nitrate-N.

Parallel experiments carried out with Hiatt Baker soil gave similar results. Following the same preliminary treatment with glutamate, the nitrification of ammonium sulphate yielded approximately 80 per cent of nitrite or nitrate after eight days' percolation, while with glutamate only approximately 40 per cent could be recovered on completion of the nitrification of ammonia.

In attempting to account for the low yields of nitrite and nitrate from glutamate in these enriched soils, there are two main possibilities. One is that immobilisation may become more active owing to the proliferation of micro-organisms and their assimilation of nitrogen during the breakdown of the amino acid. Alternatively, loss of nitrogen from the soil and percolate may occur owing to denitrification or other processes. An attempt was made to dis-

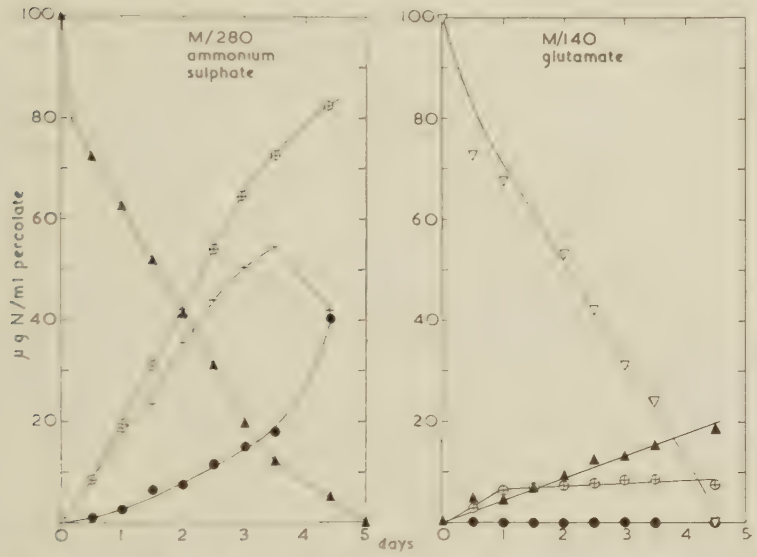

Fig. 14. Conversion of ammonium sulphate and glutamate in Brislington soil following nitrite accumulation. For each treatment mean results from three replicate samples of 10 g are shown.

Previous treatment of soil: percolation with *M*/280 glutamate until ammonification complete, followed by percolation with *M*/140 glutamate until all ammonia was converted to nitrite.

Symbols: Amino-N ▽, Ammonium-N ▲
Nitrite-N +, Nitrate-N ●
Nitrite + nitrate-N ⊕

criminate between these possibilities by analyses for total nitrogen in the soils and percolates. At the conclusion of experiments such as those described above, samples of the soil and of the percolate were analysed for total nitrogen. It was hoped to determine in this way whether the low recoveries of nitrogen could be related to retention and immobilisation of nitrogen in the soil and percolate or to losses from the system.

The soils percolated with either ammonium sulphate or with glutamate were analysed in the same way, so that a direct comparison could be made. The results of a series of analyses on Brislington soil are summarized in Table VI. In the replicate samples there is consistent evidence of a loss of nitrogen from the soils percolated with glutamate, as compared with those treated with a similar quantity of ammonium sulphate, the mean difference amounting

to 5.6 mg of total N per unit. There is also direct evidence of an increased immobilisation of nitrogen in soils treated with glutamate, amounting in this instance to a mean of 2.0 mg N per 10 g sample of soil. It is clear, therefore, that the additional immobilisation together with the loss of nitrogen largely account for the very low recovery of nitrogen in the soils percolated with glutamate.

TABLE VI

Changes of total N (mg N/unit) in enriched Brislington soil percolated with glutamate or ammonium sulphate

Treatment	Glutamate				Ammonium sulphate			
Units no.:	1	2	3	Mean	1	2	3	Mean
Soil–N	15.7	16.1	15.2	15.7	13.6	12.9	14.5	13.7
Percolate–N	5.6	2.6	4.8	4.3	11.5	11.5	12.6	11.9
Total–N	21.3	18.7	20.0	20.0	25.1	24.4	27.1	25.6

The possibility of losses of ammonia from the soil was investigated by passing the airstream from the percolating units through dilute sulphuric acid and periodically analysing the acid for free ammonia. Very little release of ammonia was detected and it is unlikely, therefore, that extensive losses of nitrogen could be accounted for in this way.

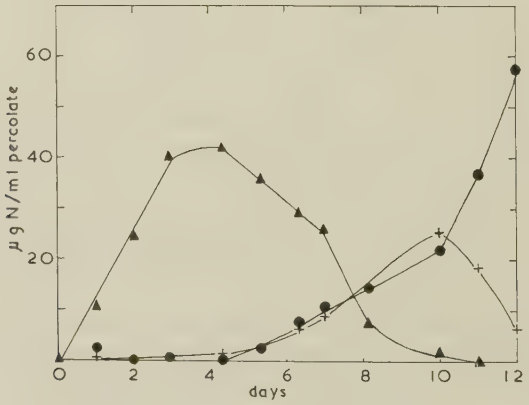

Fig. 15. Conversion of glutamate to nitrate in three replicate samples of enriched Long Ashton soil. Mean results are shown.
Previous treatment of soil: percolation with $M/140$ glutamate until nitrification complete.
Symbols: Ammonium-N ▲, Nitrite-N +
Nitrate-N ●

In addition to this direct evidence of loss of nitrogen from the soil, several other features of the nitrification processes associated with the breakdown of amino acids suggest that denitrification may be important in these fresh soils. As already shown with the Hiatt Baker and Brislington soils percolated with glutamate, there is often a prolonged lag in nitrite and nitrate production associated with a low recovery of nitrogen, which could be attributed to denitrification. Similar evidence was obtained with Long Ashton soil after enrichment with ammonifying and nitrifying micro-organisms. The second percolation of this enriched soil with glutamate gave a lower recovery of nitrate than that obtained from the first percolation (Fig. 15, compare with Fig. 8) and, as found in Brislington and Hiatt Baker soils, this is associated with a prolonged lag phase.

DISCUSSION

All the experiments in which fresh soils were percolated with glutamate or glycine have shown that ammonification proceeds rapidly. There is direct evidence both from the acceleration of ammonification and the changes in the microflora that an enrichment of heterotrophic micro-organisms occurs and that this takes place mainly on the soil crumbs.

The different soils were much more variable with regard to their capacity to promote nitrification; only three of them developed high rates of nitrification after prolonged percolation with dilute ammonium-sulphate solutions. These latter soils were relatively alkaline and calcareous; the favourable effects of such alkaline conditions on nitrification are already well established [12]. However, not all of the calcareous soils which were examined developed high rates of nitrification. The chalk grassland soil, collected from the Marlborough Downs, is one such example (Table IV). The failure of nitrification in these soils is not at present understood; it cannot be correlated with their capacity to bind ammonia by base exchange.

As shown by Quastel and Scholefield [12], the liberation of hydrogen ions during nitrification in slightly acid soils may have an important effect on the process. There were similar indications in the experiments described here with the slightly acid Long Ashton soil. With some soils examined, however, there was little or no

nitrification of ammonia released from an amino acid even after prolonged percolation. It is unlikely that the unfavourable hydrogen-ion concentration in these soils was entirely responsible for the failure of nitrification.

It is probable that the high rates of ammonification and nitrification observed in percolated soils represent the potentialities of the soil to promote these changes under favourable conditions, rather than the normal rates under field conditions. However, the greater ease and rapidity of ammonification compared with nitrification in many of the semi-natural soils raises the possibility that ammonia rather than nitrate may be the chief product of the breakdown of organic matter in these soils and that ammonium ions may be more readily available for assimilation by higher plants.

The course of ammonification and nitrification in soils percolated with amino acids is fully consistent with the hypothesis of successive stages of breakdown, first by deamination to ammonia and then by oxidation to nitrite and nitrate, as discussed by Quastel and Scholefield [12]. In detail, however, the processes in fresh soils are dependent on the type of soil and the nature and amount of amino acid with which it is percolated. Depending on the type of soil, the rate of nitrification may be either greater or less than the rate of nitrification of an ammonium salt. With Long Ashton soil percolation with glutamate leads to more rapid nitrification than with ammonium sulphate, probably owing to a favourable change in pH. In contrast, with Brislington and Hiatt Baker soils lower rates of nitrification from glutamate were obtained, associated with reduced yields of nitrate. In this respect the results differ from those reported by Quastel and Scholefield [12], who state that the rates of nitrate formation from such amino acids as glycine, alanine and glutamic acid in a slightly alkaline soil do not differ markedly from that with comparable solutions of ammonium chloride. It is possible that these differences may be caused by the preliminary treatment of the soil. It has been shown that drying, as used by Quastel and Scholefield, has a marked selective effect, particularly on the heterotrophic micro-organisms, and it is possible that the different results obtained in the present investigation may be due to the use of fresh, undried soils. Much of the evidence obtained suggests that the reduced yields of nitrate may, in part, be owing to assimilation by proliferating micro-organisms and this

will be determined to a large extent by the heterotrophic micro-organisms present initially in the soil. The extent of immobilisation is also influenced by the nature of the amino acid. A direct comparison between glycine and glutamic acid which was made in some experiments indicates the importance of the C N ratio in this respect.

A clear example of the importance of both the type and amount of amino acid in determining the course of nitrification is shown in those experiments with Brislington and Hiatt Baker soils subjected to more prolonged percolation with glutamate, which resulted in an accumulation of nitrite (Fig. 12). It is not possible to decide with certainty whether this condition results from a direct action of the amino acid on the proliferation of the nitrate-forming bacteria, although the very active formation of nitrite and the slow production of nitrate when these enriched soils are percolated with an ammonium salt supports this view (Fig. 14). It is probable, however, that the presence of the amino acid has other effects on the activity of the soil micro-organisms. Such readily available organic matter may promote the reduction of nitrate to nitrite or influence the nitrification processes by altering the level of CO_2 in the soil.

There is also evidence that denitrification may be responsible, in part, for the low yields of nitrate obtained from amino acids in some of the experiments described. Analyses indicate that, under extreme circumstances, nearly 25 per cent of the nitrogen of an amino acid may be lost from the soil and percolate (Table VI). It is now known that organisms capable of denitrification under relatively aerobic conditions are widely distributed in soils [4 9], and these may be important in nitrification processes. Corbet and Wooldridge [9] postulate three essential conditions for aerobic denitrification,

(i) a readily available supply of carbonaceous material,
(ii) nitrite and/or nitrate as a source of nitrogen and
(iii) presence of the effective micro-organisms.

The first two requirements are fulfilled during the percolation of the amino acid and the subsequent nitrification of the ammonia produced from its breakdown. Much of the evidence presented here suggests that a population of denitrifying micro-organisms is readily built up during the breakdown of amino acids in soils.

The percolation techniques offer an opportunity for studying under controlled conditions aerobic denitrification in soil systems. This is an aspect of the circulation of nitrogen on which little exact information is available, despite its important bearing on soil fertility. The results of an investigation on aerobic denitrification and its relation to soil nitrification processes will be given in a further paper.

SUMMARY

Amino-acid breakdown and nitrification were studied in cultivated and natural soils by means of a percolation technique. This was devised so that fresh soils could be used.

Ammonification proceeded rapidly in fresh soils percolated with dilute solutions of glutamate and glycine. There was evidence both from the acceleration of ammonification and bacterial counts that an enrichment of heterotrophic micro-organisms occurred and that this took place mainly in the soil.

The soils were much more variable with regard to their ability to promote nitrification when percolated with dilute solutions of ammonium sulphate.

The course of nitrification following ammonification in soils percolated with amino acids indicated successive stages of breakdown, first to ammonia and then by oxidation to nitrite and nitrate. In detail the processes varied with the type of soil and the amount of amino acid used. In one slightly acid soil (Long Ashton), percolation with glutamate gave more rapid nitrification than percolation with ammonium sulphate; in two other soils (Brislington and Hiatt Baker) lower rates were obtained. Prolonged percolation with larger amounts of glutamate had a marked effect on subsequent nitrification, most of the products of nitrification appearing in the percolate as nitrite and not as nitrate.

In soils percolated with glycine and allowed to nitrify, subsequent percolation with the amino acid lead to a rapid conversion of the amino-N to nitrate, indicating an enrichment with both ammonifying and nitrifying micro-organisms. In similar experiments with glutamate, low recoveries of nitrate were obtained. These low recoveries could be attributed to increased assimilation of nitrogen by micro-organisms and to losses of nitrogen by denitrification.

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EXCHANGE CAPACITY OF PLANT ROOTS *

I. FACTORS AFFECTING THE TITRATION VALUE

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INTRODUCTION

The property of cation exchange of root systems had been first reported by Deveaux in 1916. He attributed the phenomenon to the pectose in the walls of the root hairs. Several workers advanced postulations regarding the compounds responsibile for cation adsorption. Teorell [12] believed that in membranes, carboxylic and/or amino groups are fixed as immobile members of the main valency chains. Osterhout [10] believed that the plant substance responsibile for cation adsorption may be similar to some aromatic alcohols. Brooks [2] believed it to be amino acids. According to Lundegårdh [7], the living surface of the epidermal cells contain substances dissociating H^+ and OH^- ions, and the root surface acquires a negative potential because of the strong dissociation of the acid groups. Lundegårdh [7] believed that these groups could be radicals of phosphoric acid. Steward and Street [11] suggested that energy-rich phosphorylated nitrogen compounds may be formed at the outer plasmatic surface of cells. It is suggested that such compounds may be able to escort cations and anions across the protoplast. Mattson *et al.* [8] showed that pectic substances in the root epidermal cells in peas were responsible for the cation exchange capacity of roots. According to Frey-Wyssling [5] the structure of the plant cell shows that a protein framework extends from the cell wall and in the cytoplasm. The protein framework is built more densely close to the wall. The chemical compo-

* Part of a thesis submitted by the senior author for the degree of Doctor of Philosophy in Soil Science.

sition of the cell wall does not remain constant as the cell advances in age. Moreover, different active groups are present in both frameworks of the cytoplasm and the wall.

Whatever the nature of the groups on the root surface may be, it is found that H^+ ions produced by metabolic processes are present on the surface of the plant root. These H^+ ions are readily exchanged by other metalic cations such as Ca^{++}, K^+ ... as shown by Williams and Coleman [14] and by Graham and Baker [6]. Furthermore these H^+ ions lend themselves to quantitative determination.

The total cations which can be replaced under a given set of conditions is known as the cation exchange capacity of the root (C.E.C.) and is expressed usually as me per 100 g of roots.

The values obtained for C.E.C. of plant roots by titration are susceptible to variations according to the conditions prevailing during determination.

The present investigation deals with the study of certain aspects of the cation exchange capacity of some plant roots and some factors influencing the titration value.

MATERIALS AND METHODS

Excised roots of barley, peas, and wheat have been used in this investigation. Seeds were germinated in tap water, continuously aerated for 24 hours. A few drops of H_2O_2 were added to enhance germination and to act as a germicide. The seeds were then transferred to galvanized screens covered with cheese cloth. Every screen held about 80 g of seeds evenly distributed. The screens were supported on enameled 4-liter trays containing tap water. The water was changed every day. The roots were usually excised after a certain period varying between 5 to 12 days. They were then subjected to certain treatments to meet the special requirements of the particular experiment as will be described briefly under each experiment.

The main features of the technique involved the washing of the excised roots with acid followed by washing with H_2O until the roots were acid free. An amount of roots varying between 2 and 3 g was titrated with standard (around 0.04 *N*) KOH or $Ba(OH)_2$ in 50 ml KCl or $BaCl_2$, to definite pH using a glass-electrode pH meter. Titration time of 2 minutes was taken as standard in all experiments except when otherwise indicated. After titration, the roots were washed with water and then dried at 70°C for 24 hours and weighed. Cation exchange capacity of roots is expressed as milliequivalents per 100 g of dry roots.

EXPERIMENTS, RESULTS AND DISCUSSION

1. SATURATION OF ROOTS WITH HYDROGEN

In the method used for the determination of cation exchange capacity, the roots should be saturated with hydrogen ions before titration. The saturation of roots is an exchange reaction affected, among other things, by the number of rinses, concentration, and type of acid used in washing the roots. In the following experiments these three factors are studied.

A. Effect of number of rinses with acid on the cation exchange capacity of roots

Barley plants (variety Hungarian) were grown for 6 days as described before. From 2- to 3-g portions of roots were washed once, twice, 3, 4, 5, 6 and 7 times with 100-ml portions of HCl at pH 2.0. Roots were left in each washing for 15 seconds. They were then washed with H_2O until freed from chloride. Potassium exchange capacity was determined as described before.

The results obtained are shown in Table I. It is seen that the cation exchange capacity of roots depends on the number of washings with acid up to 5 times. H^+ ions replaced more than 90 per cent of the cations on the surface of the roots after four rinses. Five rinses were found sufficient to completely saturate the roots with H^+ ions and remove all the cations on the surface. Therefore, five washings with 100-ml portions of HCl at pH 2.0 were used as a standard technique in the determination of the cation exchange capacity of roots during the present investigation.

TABLE I

Effect of number of rinses with HCl on the cation exchange capacity of roots

Number of rinses with acid	Cation exchange capacity me/100 g dry roots
1	5.60
2	5.83
3	6.71
4	6.82
5	7.30
6	7.37
7	7.35

B. Effect of concentration of acid on the cation exchange capacity of roots

Barley plants (var. Hungarian) were grown for 12 days as described above. Roots were excised and washed 5 times with 100-ml portions of HCl of varying normality. The roots were then washed free from acid with distilled water. K-exchange capacity was determined as described above.

TABLE II

Effect of pH of HCl on the pH and cation exchange capacity of barley roots

Conc. of acid	pH of acid	pH of roots	K.E.C.*
0.1 *N*	0.8	4.0	6.62
0.05 *N*	1.1	4.1	6.60
0.025 *N*	1.5	4.3	5.74
0.012 *N*	1.8	4.6	5.47
0.006 *N*	2.1	4.9	5.19
H_2O	5.6	5.5	1.80

* K. E. C. = Potassium exchange capacity

It is shown in Table II that as the concentration of HCl increased the pH of roots decreased. Accordingly K-capacity increased. It appears that the exchange of adsorbed cations for H^+ ions on the root surface becomes more complete as the concentration of the acid used in washing is increased. It is therefore clear that the concentration of the acid used in washing the roots has an effect on their cation exchange capacity. In determining this value, the concentration of the acid used must be clearly stated and be high enough to permit complete saturation of the roots with H^+ ions.

C. Effect of different acids with the same pH on the cation exchange capacity of roots

Different acids, including HCl, HNO_3, H_2SO_4, H_3PO_4, HAc, and H_2O were used for washing the roots. All the acids had a pH of 2.0 except HAc had a pH of 3.0 and H_2O had a pH of 5.7. The same procedure for C.E.C. determination described above was followed.

The results in Table III indicate that the acid itself had practically no effect on the cation exchange capacity of roots as long as the pH was the same. If the anion of the acid would have an effect, it is expected to appear only under equilibrium conditions. How-

ever in the present experiment no equilibrium was attained due to the successive washing.

TABLE III

Effect of different acids with the same pH on the pH and cation exchange capacity of barley roots

Acid	pH of acid	pH of root	K.E.C.
H_2O	5.7	5.6	1.54
HAc	3.0	4.9	4.52
H_3PO_4	2.0	4.4	4.35
HNO_3	2.0	4.4	4.03
H_2SO_4	2.0	4.4	4.26
HCl	2.0	4.4	4.24

Table IV shows results obtained for barley (Hungarian variety) and wheat (Giza 139 var.) roots. All were washed with H_2CO_3, and HCl solutions. The weak H_2CO_3 acid produced less saturation of roots with H^+ ions than HCl. The cation exchange capacity was lowered accordingly. These results substantiate the conclusion that the pH of acid used in washing is an important factor in determining the values of the cation exchange capacity of roots.

TABLE IV

Effect of rinsing acid on the cation exchange capacity of barley and wheat roots

Acid	pH of acid	pH of roots	K.E.C.
I*. H_2O	5.2	4.7	3.92
H_2CO_3	3.9	4.7	4.82
HCl	2.2	4.1	6.63
II*. H_2O	5.2	4.2	4.39
H_2CO_3	3.9	4.2	7.53
HCl	1.9	4.2	7.93

* I = Barley Hungarian var., 7 days old.
II = Wheat Giza 139 var., 6 days old.

2. TITRATION OF PLANT ROOTS

A. General behavior during titration

Titration curves were made both on fresh and killed roots. Fresh roots were obtained and saturated with H^+ ions in the same way as described before. From 2–3 g of roots were titrated with standard KOH in 50 ml of 1 *N* KCl. Another sample of 2–3 g of fresh roots was titrated in 50 ml 1 *N*

$BaCl_2$ with standard $Ba(OH)_2$. After each addition of alkali, the pH was determined by a glass-electrode pH meter. After completion of titration, the roots were washed with water, dried at 20°C for 24 hours and weighed. Other portions of roots were boiled in H_2O at 100°C for 5 minutes. They were then washed 5 times with 100-ml portions of HCl at pH 2.0, then washed free from acid with water. The titration and subsequent treatments were the same as with fresh roots. Titrations were made for barley (var. Hungarian) 12 days old.

The results are shown in Table V and plotted in Figure 1. It

TABLE V

Titration curves of fresh and boiled barley roots.

Root state	pH	me KOH per 100 g dry root	pH	me Ba $(OH)_2$ per 100 g dry root
Fresh	3.6	0.00	3.6	0.00
	3.9	2.51	3.9	2.63
	4.4	5.02	5.1	5.39
	5.6	8.78	7.8	10.52
	7.0	11.29	8.3	12.49
	7.8	13.80	8.6	14.46
	8.6	17.56	8.9	17.09
	9.1	21.33	9.3	21.04
	9.3	25.09	9.5	27.61
	9.6	33.87	9.8	32.87
	9.8	37.63	9.9	36.82
	10.0	38.92	10.0	39.45
Boiled	3.6	0.00	3.6	0.00
	4.1	2.76	4.1	2.62
	4.8	5.51	5.3	5.24
	6.3	8.27	7.4	7.86
	8.6	11.02	8.7	10.48
	9.2	13.78	9.5	13.10
	9.5	16.54	9.9	15.72
	10.0	19.29	10.0	18.34

appears from the data obtained that the pH of the roots at the beginning represented an acid of a weak nature. However, the first part of the curves showed much greater change than observed with regular acids. The amount of alkali required to cause a change in pH was about the same for both fresh and killed roots up to pH 6.0. This was also observed by Williams and Coleman [14] and led them to conclude that the exchange capacity of roots is not related to metabolism, or it is associated with compounds which do not change immediately after the cessation of metabolism. The

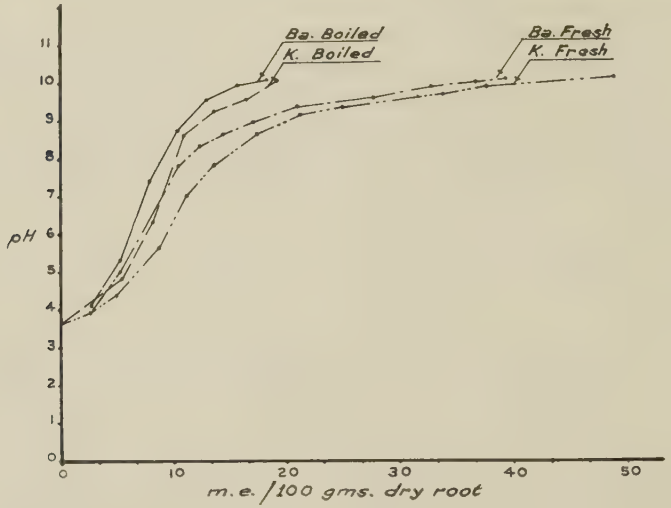

Fig. 1. Titration curves of fresh and boiled barley roots with KOH and Ba $(OH)_2$.

difference between fresh and killed roots began to show itself more clearly above pH 6.0. At higher pH values the amount of alkali required to cause change in pH was greater for fresh than for killed roots. Buffering effect was more pronounced in the case of fresh roots. The curve of killed roots showed sharp changes while living roots showed gradual changes. The equivalence points fell between pH 7 and 8. This may indicate the presence of a salt of a weak acid (root) and strong base (KOH or $Ba(OH)_2$), and explains why the cation exchange capacity is determined by titration to pH around 8.0.

Curves for both fresh and killed roots are similar to those of monobasic acids, although they are not true ones.

There is a quick replacement of H^+ ions from root surface by other cations. This is in accord with the findings of William and Coleman [14], who concluded that the titration curves of roots are similar to a titration curve of a strong acid. Some of the H^+ ions on the root surface dissociate more readily than others and only at relatively high pH values do all the H^+ ions dissociate. A dependence of the cation exchange capacity of roots on the pH is, therefore, expected. Such findings are in accord with facts known about the cytoplasm of plants. According to Frey-Wyssling [5], the

dissociation of the acidic groups diminishes below the iso-electric state of the cytoplasm and increases as the pH is increased above the I.E.S. and the cytoplasm becomes negatively charged.

Mattson *et al.* [8], believed that the exchange capacity of roots remains constant between pH 5 and 9. This explains why plants tolerate wide pH ranges. They assumed that only pectic substances were involved in titration. Their conclusions are not in agreement with the data obtained here, as significant changes in the cation exchange capacity took place within this pH range.

The curve obtained with $Ba(OH)_2$ lies above that with KOH both for fresh and killed barley roots. This may be due to the greater replacing power of the Ba-ion and the weaker dissociation of the barium root.

B. Effect of concentration of salt used in the titration process on the cation exchange capacity of roots

In order to repress dissociation of adsorbed ions from the surface, titration is usually carried on in the presence of a neutral salt. Furthermore, the exchange of H^+ ions by metallic cations takes place to different extents depending upon concentration. Therefore, it was found essential to study the effect of concentration of salt used in the titration on the cation exchange capacity of roots.

Excised roots were obtained and saturated with H^+ ions by the same technique described before. 2-3 g of H-saturated roots were titrated with standard KOH to pH 8.0 in 50-ml KCl solutions of different concentrations. Similar quantities of roots were titrated with standard $Ba(OH)_2$ in $BaCl_2$ solutions of different concentrations. Titration time was two minutes. Roots were then washed with H_2O, dried, and weighed.

The results are shown in Table VI for barley (var. Hungarian) 12 days old, and for peas (var. Torsdag) 21 days old, and presented graphically for barley in Figure 2.

Exchange reactions are known to be stoichiometric and governed by the laws of mass action and chemical equilibrium. Therefore, in general, increased concentration of replacing cation causes greater exchange of the replaced cation, especially if it is removed from the reaction, *i.e.*, $H^+ + OH^- \rightarrow H_2O$.

It is observed that the amount of H^+ replaced by K^+ or Ba^+ ions increased with the increase in concentration of replacing cation

TABLE VI

Effect of concentration of salt added in the titration process on the cation exchange capacity of roots

Plant	Concentration	K.E.C.	Ba.E.C.
Barley	50 ml H_2O	3.04	4.73
	0.05 *N*	4.76	5.22
	0.1 *N*	5.47	7.14
	0.2 *N*	5.40	7.09
	1.0 *N*	5.47	7.82
Peas	50 ml H_2O	2.66	2.92
	0.01 *N*	3.10	4.29
	0.02 *N*	4.45	5.12
	0.04 *N*	4.77	5.49
	0.08 *N*	5.42	6.81
	1.0 *N*	5.50	6.82

up to a concentration of 0.1 *N*. At higher concentrations no further exchange took place. It is noteworthy that such concentrations represent several hundreds of symmetry concentrations with respect to the exchange capacity of the roots used in the titration. More H^+ ions were replaced by Ba^+ than by K^+ ions in both barley and pea roots. The type of curves obtained here with roots are similar to those obtained usually with other exchange materials and soils and known as adsorption isotherms.

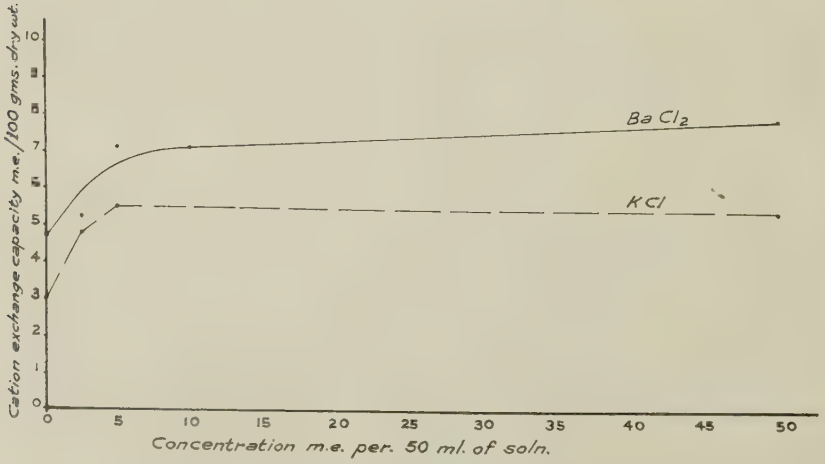

Fig. 2. Relation between cation exchange capacity of barley roots and concentration of replacing cation.

It could be concluded that in determining the cation exchange capacity of roots by titration in a salt solution, the concentration of the salt must be stated and it should not be lower than 0.1 *N*. It is preferable to carry the titration in 1 *N* salt solutions with ions common to that of the alkali used.

C. Effect of valence and hydration of cations of the salt solution on the exchange capacity of roots

The extent of adsorption of cations on surfaces are usually governed by their valence and hydration. This behaviour is observed on the surface of clays and other exchange materials. To examine such behaviour with root surfaces, excised and H-saturated roots were obtained by the same technique as described before. 2–3 g of roots were titrated to pH 8.0 with standard KOH or $Ba(OH)_2$ in 1 *N* chloride solutions of the alkali and alkali earth cations. Titration time was 2 minutes, after which the roots were washed with H_2O, dried at 70°C for 24 hours, and weighed. The results for barley (var. Hungarian) 5 days old, and for wheat (var. Giza 139) 5 days old are shown in Table VII.

TABLE VII

Effect of valence and hydration of cations of salt solutions on the cation exchange capacity of barley and wheat roots. (time of reaction — 2 minutes)

Solution (1*N*, pH 8.0)	Hydroxide used	Cation exchange capacity	
		Barley	Wheat
LiCl	KOH	5.86	7.21
NaCl	KOH	8.03	8.96
KCl	KOH	4.94	8.08
$MgCl_2$	KOH	6.80	13.18
$CaCl_2$	KOH	9.21	15.62
$SrCl_2$	KOH	8.31	10.14
$BaCl_2$	KOH	5.16	6.99
NaCl	$Ba(OH)_2$	6.14	—
KCl	$Ba(OH)_2$	5.23	7.32
$MgCl_2$	$Ba(OH)_2$	7.13	—
$BaCl_2$	$Ba(OH)_2$	5.32	8.78

Another experiment with fresh and killed roots of barley 4 days old was carried out in the same way as above. In this case titration

to pH 7.0 was carried out in 0.1 *N* solutions after an equilibrium time of 20 minutes. Killed roots were obtained after boiling in H_2O at 100°C for five minutes. Roots were then washed 5 times with 100-ml portions of HCl at pH 2.0, then with H_2O until they became acid free. The results are shown in Table VIII.

TABLE VIII

Effect of valence and hydration of cations of salt solutions on the cation exchange capacity of fresh and killed barley roots. (Time of reaction –20 minutes)

Solution (0.1 N, pH 7)	Cation exchange capacity	
	Fresh roots	Killed roots
LiCl	4.92	4.97
NaCl	5.83	4.13
KCl	5.43	4.14
$MgCl_2$	5.21	3.72
$CaCl_2$	5.21	4.54
$SrCl_2$	5.24	3.77
$BaCl_2$	5.56	4.39

From the above experiments, there seems to be no definite lyotropic order followed in the exchange reaction with roots for ions of similar valence. This may be explained by the specificity of certain adsorption spots for the different cations.

Epstein and Hagen [4], working with barley roots, showed that K^+, Rb^+, and Cs^+ are absorbed by the same sites but not Na^+. However, Williams and Coleman, [14] working with H^+-saturated roots of tobacco, were able to show the usual order of lyotropic affinities at different concentrations. Even in clays and other exchange materials, the lyotropic order is affected, among other things, by concentration, replaceable ions, and degree of saturation. Wiklander [13], working with different exchange materials had shown that there was no single definite order of the replacing power of cations.

D. Effect of time of reaction on the cation exchange capacity of roots

The study of reaction rate gives valuable information about the nature and way by which reactions take place.

For this study, excised and H-saturated fresh pea roots 21 days old (var. Torsdag), fresh and killed roots of barley (var. Hungarian) 21 days

old, were obtained by the same technique described before. 2- to 3-g portions of roots were put in 1 *N* KCl or $BaCl_2$ solutions of pH 8.0. After 2 minutes the pH was measured by a glass-electrode pH meter and found to have fallen below pH 8.0. Standard hydroxide solutions of K or Ba were added to bring the pH back to 8.0. The amount of hydroxide taken to bring solutions to pH 8.0, calculated as me per 100 g dry weight of roots were taken as the cation exchange capacity of roots after the given time period. The process was repeated after several periods of time as shown in Table IX. Finally, the roots were washed with H_2O, dried at 70°C for 24 hours, and weighed.

The results are shown in Table IX and presented in Figure 3. From the data presented in Table IX, it appears that the exchange reaction with roots requires relatively short time. This fact was also observed by Mattson *et al.* [8], by Williams and Coleman [14], and by Mclean and Baker [9]. With fresh and killed barley roots as well as with roots of peas, both K and Ba exchange capacity increased with time. This behaviour was also noted by Mattson *et al.* [8] and by Drake *et al.* [3] who adopted a standard time of 5 minutes as a titration time.

TABLE IX

Effect of time of reaction on the cation exchange capacity of pea roots, fresh and killed barley roots

Plant material	Time in minutes								
	2	5	10	20	30	60	90	120	180
Barley (*Fresh roots*)									
K.E.C.	5.27	7.35	8.89	9.87	10.31	11.30	12.29	13.28	14.26
Ba.E.C.	5.86	7.82	9.16	10.14	10.75	11.97	12.95	13.93	14.91
Barley (*Killed roots*)									
K.E.C.	2.66	4.26	4.79	5.32	5.86	6.39	6.92	7.32	
Ba.E.C.	2.76	4.42	4.84	5.25	5.67	6.08	6.49	6.91	
Peas (*Fresh roots*)									
K.E.C.	6.27	8.71	12.23	14.21	16.20	18.34			
Ba.E.C.	7.45	10.43	14.53	18.07	24.03	25.33			

After a period of one hour, an approximately 3-fold increase in exchange capacity of the roots studied was observed. Such an increase was also observed by Williams and Coleman [14], who observed that the exchange capacity of fresh barley roots was 3 times as great after one hour as after 30 seconds. From Figure 3 it seems

that a large portion of hydrogen ions is released by exchange in the first 10 minutes as shown by the very steep rise in the curves. After this time a constant rate of releasing hydrogen from the surface of roots took place. This could be explained on the basis that different groups are present on the surface of the root and take part in the exchange reaction. The strong-dissociating groups are easily neutralized at first, and are followed by the less-dissociating groups. Such behaviour is usually observed with ion exchange resins as discussed by Bauman [1]. A resin containing different groups, as $-SO_3H$, -COOH, and phenolic -OH groups, in the same structure, when titrated with a hydroxide will show instantaneous neutralization of the strong $-SO_3H$ groups, a 2- to 4-hours time to obtain equilibrium with COOH groups, and a 24-hours drift to obtain equilibrium with phenolic -OH groups.

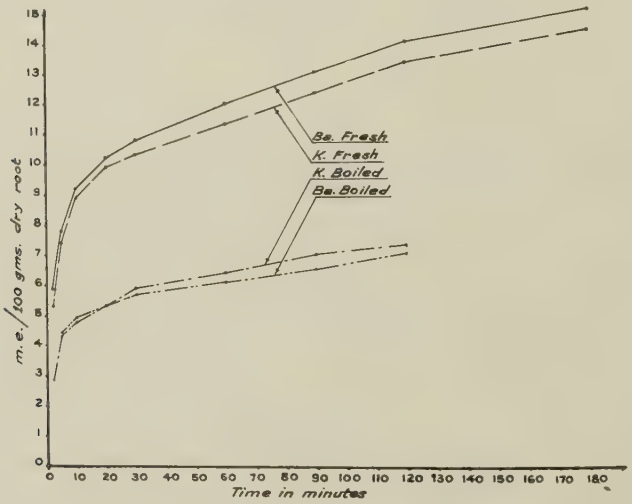

Fig. 3. Variation of cation exchange capacity of fresh and boiled barley roots with time.

The curve of killed barley roots, Figure 3, was much below that of fresh roots, but the same trend of fresh roots of peas and barley were observed.

The exchange reaction with roots is a surface reaction and proceeds rapidly. It takes place practically as soon as contact is established between solution and surface. However, a true equilibrium is

difficult to reach, since according to the equation

$$R\text{–}H + B^+ \underset{K_2}{\overset{K_1}{\rightleftharpoons}} R\text{–}B + H^+$$

the equilibrium constants of the direct and reverse reaction may be different. The rate also depends on whether replacing cations travel longer distances or take places on outer surfaces. Exchange reactions at deep-seated surfaces require longer times to reach equilibrium. The rate depends on whether or not several types of groups are involved. Short intervals are required with strong groups, while longer intervals are required for weak-dissociating groups. The higher values obtained with longer periods of time, may include, in addition to the part played by the different exchange spots present on the surface, ion uptake with the result of continuous release and outgo of H^+ ions.

SUMMARY

A study of some factors affecting the titration value of plant roots has been carried out.

1. Factors affecting the saturation of roots with H^+ ions have been studied. These factors include number of rinses with acid, concentration, and type of acid.
   a. Five rinses were found sufficient to remove most of the cations from the root surface, and this was taken as a standard procedure.
   b. The concentration of the acid used in rinsing was found to influence both pH and C.E.C. of roots up to a certain extent. As the concentration of HCl increased, the pH of the roots decreased and their cation exchange capacity increased. Therefore the concentration of the acid must be standardized.
   c. The anion associated with acid used in washing the roots had practically no effect on the C.E.C. of roots when compared at equal pH.
2. Titration curves of roots and factors influencing them, including fresh and killed, and presence of salts, time of reaction, and valence effect have been studied.
   a. Curves of fresh and killed roots were the same up to pH 6 and then differed greatly as titration proceeded. Buffering was more pronounced with fresh roots. The inflection point occurred between pH 7 and 8. Curves obtained with $Ba(OH)_2$ were always above those with KOH, both for fresh and killed roots.
   b. Titration in the presence of a salt with a common cation to the alkali added is influenced by the concentration of the salt up to 0.1 *N*. The amount of alkali required for titration increased as the concentration of salt increased.

c. Effect of valence and hydration of alkali and alkali earth cations on the C.E.C. of roots were studied. No definite lyotropic order was observed with ions of equal valence. Divalent cations generally had stronger replacing power than monovalent.

d. The effect of time of reaction on the cation exchange reaction with roots was studied. The exchange reactions with roots is a quick reaction. The amount of alkali required for titration increased with time. Most of the H^+ ions on root surface were replaced during the first 10 minutes, after which a constant rate of releasing H^+ ions took place. This may indicate the presence of different acid groupes as well as intake of ions with time.

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EXCHANGE CAPACITY OF PLANT ROOTS *

II. SOME FACTORS AFFECTING THE CATION EXCHANGE CAPACITY.

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INTRODUCTION

Several workers emphasized the importance of the property of cation exchange of plant roots. Jenny and Overstreet [8] believed that the exchange properties of the root enables the plant through contact exchange to feed directly on the solid phase of the soil. Elgabaly and Wiklander [4] showed that the differential uptake of cations of different valences by roots having different exchange capacity acts in favour of the importance of exchange capacity of roots in plant feeding. Burstrom [2] believes that the first step in ion accumulation is adsorption on the surface of plasma colloids. It is expected therefore, that the study of the nature of the property of cation exchange of roots and the factors affecting and controlling it, is of great importance in plant nutrition.

The present investigation is a study of certain factors which seem to affect the cation exchange capacity of plant roots.

These factors studied include age of plant, nitrogen content of roots, growth inhibitors and stimulators, and the effect of certain solvents.

EXPERIMENTS, RESULTS AND DISCUSSION

A. The relation between cation exchange capacity of roots and age of plant

Several properties of plant roots change as the plant advances

* Part of thesis submitted by the senior author in partial requirements for the degree Doctor of Philosophy in Soil Science.

** Assistant and Professor of Soil Science respectively.

in age, Of these, ion uptake is an important one. This is probably associated with the cation exchange capacity of roots. In the following experiment the effect of age on the C.E.C. of barley roots is studied over a period of 12 days.

Seeds of barley (Von Variety) were germinated in tap water aerated continuously for 24 hours. Few drops of H_2O_2 were added to enhance germination and to act as a germicide. The seeds were then transferred to galvanized screens covered with cheese cloth. Every screen held about 80 g of seeds evenly distributed. The screens were supported on enameled 4-liter trays containing tap water. The water was changed every day. Portions of the roots were excised at 3 days intervals and used for the determination of the cation exchange capacity. The excised roots were washed 5 times with HCl at pH 2.0, followed by washing with H_2O until the roots were chloride free. 2 to 3 g of roots were titrated with standard (around 0.04 *N*) KOH in 50 ml of *N* KCl to pH 8.0 using a glass-electrode pH meter. Titration time was two minutes. After titration, the roots were washed with water and dried at 70°C for 24 hours and weighed. Cation exchange capacity is expressed as me per 100 g of dry roots. Titrable acidity of roots was determined by boiling the roots at 100°C in H_2O for 5 minutes and titration of total acidity with standard KOH to pH 8.0.

TABLE I

The change of cation exchange capacity of barley roots with age of plant

Age of roots, days	K.E.C.* me/100 g	Titrable acidity me/100 g	K.E.C./ Acidity
3	7.77	38.00	0.20
6	5.04	24.53	0.21
9	4.68	20.80	0.23
12	3.85	16.61	0.23

* K-exchange capacity.

From Table I it is shown that as barley plants advanced in age, the K-exchange capacity of roots decreased rapidly at first and then more gradually. Acids in the roots showed a parallel behaviour. Graham and Baker [6] showed that the cation exchange capacity of barley roots decreased with age. Reder [11] showed that ether extractives decreased as plant matures, in 14 types of grasses. Bonner [1] discussed the chemical composition of plant cell walls. Their composition varies as the cell advances in age. Pectic substances constitute about 50 per cent of the cell-wall material in

young tissues, but decreased to only 0.5–1.5 per cent as the tissues matured. Cellulose, lignin, pentosans and similar material increased accordingly. These materials seem to be inactive with respect to cation exchange properties while pectic substances are active in this respect.

According to Jacobson and Ordin [7] organic acids play an important role in plant respiration and malic acid is the most important in barley to compensate in ion balancing. While both acids and cation exchange capacity of roots decreased with age, yet the ratio K.E.C. acids remained almost constant. This may throw some light on the part which the acids may play in the process of cation exchange of roots.

The decrease in C.E.C. of barley roots with age may further be explained on the basis that the ratio of the highly active root tips to the relatively inactive part of the root becomes smaller as the root grows older.

B. Nitrogen–root exchange capacity relationships

Several workers [10 12] reported that the exchange capacity of roots is affected by their nitrogen content. In the following experiment the effect of different concentrations of NH_4Cl in the media on the exchange capacity of roots is studied.

Barley plants (Var. Hungarian) were grown for 10 days by the same technique described above. NH_4Cl was added in 40, 20 and 10 me per 3.5 liters of tap water, 48 hours before the roots were excised. A control was grown in tap water. The cation exchange capacity was determined by titration of H-saturated roots in 1 *N* KCl or $BaCl_2$ with standard KOH or $Ba(OH)_2$ to pH 8.0 as described above. Total nitrogen content of roots was determined by micro Kjeldahl method.

It is seen from Table II that as the concentration of NH_4Cl increased in the media the nitrogen content of the roots and their cation exchange capacity increased. However the increase in cation exchange capacity is not proportional to the increase in total nitrogen. Mclean *et al.*[10] found high correlation between the cation exchange capacity of roots and their nitrogen content. They showed that the cation exchange capacity of roots and their N content were not two parallel quantities, but the relation was rather curvilinear and that the type of plant influenced the values obtained. Smith

and Wallace [12] showed that nitrogen fertilization increased cation exchange capacity of plants with low C.E.C. much more than it did with plants with high C.E.C.

TABLE II

Effect of the amount of NH_4Cl in the media on the cation exchange capacity and nitrogen content of barley roots (me per 100 g dry weight)

NH_4Cl me	pH of media	pH of roots	K.E.C.	Ba.E.C.	N content
40	5.9	3.9	5.64	7.60	211.8
20	6.3	4.0	4.90	5.97	207.4
10	6.9	4.1	4.90	5.56	190.0
Tap water	7.4	4.3	4.44	4.64	181.2

The influence of nitrogen salts on the exchange capacity may be related to the formation of energy-rich phosphorylated nitrogen compounds at the outer surface of cells. Such substances may be able to escort cations and anions across the protoplast as suggested by Steward and Street [13].

C. Effect of inhibitors on the cation exchange capacity of roots

If the cation exchange capacity of roots is related to metabolic activity of living cell, growth inhibitors may have an effect on this property.

To study the effect of inhibitors, barley plants were grown for 5 days by the same technique as described above. Portions of the roots were excised and put in solutions containing NaCN, NaN_3 and Na_3AsO_3 in concentrations varying between 0.01 and 0.001 *N*. After 2 hours the roots were removed and their K-exchange capacity determined as described before.

The results are shown in Table III.

Cyanide and azide are known as respiration inhibitors [1]. They also inhibit peptide-bond formation through their action on enzymes, especially those enzymes containing heavy metals. Both cyanide and arsenite inhibit the action of growth substances. Arsenite also interferes with phosphorous metabolism [1 3]. If such compounds affect the cation exchange capacity of roots, it should be a strong evidence in favour of the dependent nature of this property on the metabolic activity of living cells. The data presented in Table IV throw some light in this respect. At concen-

tration of 0.01 *N* a marked decrease in K.E.C. relative to control was observed with the three compounds studied.

TABLE III

Effect of different concentrations of NaCN, NaN_3, and Na_3AsO_3 on the potassium exchange capacity of barley roots

Concentration	K.E.C. in the presence of		
N	NaCN	NaN_3	Na_3AsO_3
0.010	4.08	5.64	4.08
0.008	5.02	5.64	6.26
0.006	5.02	5.80	6.42
0.004	5.07	5.71	6.80
0.002	5.57	5.70	6.80
0.001	5.86	5.72	6.49
H_2O	6.36	6.55	6.84

We can conclude that, at least part of the cation exchange property of the root presents itself as a metabolic dependent property.

D. Effect of 2,4–dichlorophenoxyacetic acid on cation exchange capacity of barley roots

We have shown in the previous experiment that some metabolism inhibitors had a reducing effect on the C.E.C. of roots. Now, it is of interest to study the effect of growth promotors on this property.

Six- and eight-day old barley plants (var. Hungarian) were grown in tap water by the same method as described before. Sodium salt of 2,4-D was added to the media 48 hours before excising the roots. Two concentrations including 8 and 16 mg of 2,4-D per 3.5 liters of media were used. K- and Ba-exchange capacity of roots were determined as described before.

The results obtained were as follows:

Treatment	K.E.C.		Ba.E.C.	
	6 days old	8 days old	6 days old	8 days old
8 mg 2,4–D	7.76	7.04	7.97	7.73
16 mg 2,4–D	5.20	4.41	5.7	4.91
Tap water	7.49	6.55	7.87	6.63

The exact nature of the mechanism of 2,4–dichlorophenoxy-acetic acid on growth is not known. But it is known generally [1], that its effect is through the respiratory process. Among its effects is the promotion of cell division, cell elongation, and new root formation.

However, at high concentrations it may produce the reverse action on plant growth as it inhibits elongation and growth of roots and retards flower formation. Epstein [5] believes that 2,4-D promotes growth and therefore, provides additional exchange spots and increases cation exchange capacity of tissue. The results obtained show that at the lower concentration, 2,4-D increased the cation exchange capacity of barley roots whereas at the higher concentration it caused a decrease in the C.E.C. It was also observed that at the high concentration the roots were brownish in colour and shorter than those grown in tap water.

E. Effect of solvents on the cation exchange capacity of roots

In order to understand the nature of the adsorption spots and their stability, an experiment was done with some solvents as described below.

Barley plants (var. Hungarian) were grown for 8 days by the same method as described before. The roots were excised and thoroughly mixed. K-exchange capacity of fresh and boiled roots were determined separately by the usual method. Samples of roots were placed in absolute alcohol and others in ether. The samples were removed from alcohol or ether after 1, 5, 10, 30, 60, and 120 minutes. The roots were immediately washed with water, saturated with H^+ and their cation exchange capacity determined by the usual method. Exchange capacity of boiled roots was 3.35 me per 100 g dry weight.

TABLE IV

Effect of immersion in alcohol and ether for different times on the cation exchange capacity of barley roots

Time of immersion (minutes)	K.E.C.	
	Alcohol series	Ether series
0	8.03	8.03
1	7.84	7.55
5	7.83	6.07
15	7.68	5.85
30	7.65	5.38
60	6.78	4.35
120	5.09	4.32

From Table IV it is seen that K exchange capacity of barley roots decreased continuously with time of immersion, both in alcohol and ether. In alcohol, about 2 per cent decrease in C.E.C. took place after one minute and 37 per cent after two hours;

whereas in ether about 6 per cent decrease after one minute and 46 per cent after 2 hours. This indicates that ether had a greater effect than alcohol in decreasing the C.E.C. of barley roots. It is possible that immersion in these solvents causes partial solution of groups responsible for cation exchange. After long periods both killing and solution may take place. Boiling the roots in water for 5 minutes at 100°C decreased the K.E.C. by about 58 per cent.

It seems that part of the groups responsible for the cation exchange capacity of roots consists of labile compounds easily destroyed or dissolved, while other groups are more resistant to solvents and boiling.

However, Williams and Coleman [14] showed that living roots and roots killed by immersion in ether had about the same C.E.C. They, therefore, concluded that the cation exchange properties of roots are associated with non-living portions of the root.

SUMMARY

Some factors affecting the cation exchange capacity of roots have been studied:

a. The change of cation exchange capacity with age was studied. Cation exchange capacity and acids in the roots decreased with age. A relation seems to exist between C.E.C. and acid content of roots.

b. The relation between C.E.C. of roots and nitrogen was studied. C.E.C. and total nitrogen content of roots increased with increasing nitrogen in the media.

c. The effect of NaCN, NaN_3 and Na_3AsO_3 at different concentrations on C.E.C. of roots was studied. These substances seem to reduce the cation exchange capacity even at low concentration. It is concluded that at least part of the groups responsible for the C.E.C. of roots are products of metabolic processes.

d. The effect of the growth promotor 2,4-dichlorophenoxyacetic acid on the cation exchange capacity of roots has been studied. C.E.C. of barley roots increased at the lower concentration whereas it decreased with increasing the concentration above a certain level.

e. K-exchange capacity of roots decreased as a result of immersion in alcohol and ether. Ether had a greater effect than alcohol and the effect of both solvents increased with time. Boiling in water decreased C.E.C. markedly. It seems that part of the groups responsible for C.E.C. of roots are easily destroyed by killing the roots.

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